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## DETERMINATION OF THE PHYSICAL WORKING CAPACITY

A PHYSIOLOGICAL AND CLINICAL STUDY WITH  
SPECIAL REFERENCE TO STANDARDIZATION OF  
CARDIO-PULMONARY FUNCTIONAL TESTS

BY

HOLGER WÄHLBERG



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*From the Laboratory of Clinical Physiology and the Medical Clinic,  
Karolinska sjukhuset, Stockholm.*

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## P R E F A C E

This work was begun in 1944 when Docent Torgny Sjöstrand, head of the Laboratory of Clinical Physiology, encouraged me to take up investigations on working capacity, and was carried out in conjunction with the Medical clinic. In this respect I am greatly indebted to Docent Sjöstrand for his never failing assistance and advice, and to Professor Nanna Svartz, head of the Medical clinic, for her stimulating interest in my work.

My father Fil. dr. Arvid Wahlund who many years ago taught me the elements of mathematical statistics, has offered me most valuable advice in the statistical treatment of the material, and for this and the keen interest he has shown I express my heartfelt thanks.

I am particularly indebted to all my colleagues and friends who have interested themselves in the completion of my work.

For the revision of the English of my work my sincere thanks are due to Mr. Ronald Taylor.

Further my thanks are due to the Board of Professors of the Karolinska Institutet for granting me a state doctorand stipendium.

Above all I must acknowledge a deep debt of gratitude to my wife for the patience she has displayed in the preparation of the manuscript and with subsequent proof-reading.

No special case reports are included, but are available in the library of the Karolinska Institutet, Stockholm. The primary material upon which calculations have been based as well as additional numerical information is collected in the appendix at the end of this book.

Stockholm, November 1948.

*Holger Wahlund*



# INTRODUCTION

Many so called functional tests have been put forward for estimating the working capacity of cases with heart or lung diseases. A functional test can usually not differentiate between various types of heart or lung diseases, but they have often been used for diagnostic purposes, causing both under- and overestimation of their usefulness. Other clinical examinations must be used to discover the origin of a poor working ability. A reliable case history is usually sufficient for an approximate judgement of the capacity for physical work. It is however valuable, and sometimes necessary, to know the influence of heart or lung diseases upon the working ability, and to know the actual working capacity when no heart or lung symptoms are discovered in persons complaining of poor ability to work. From an ideal point of view a functional test should concern only the diseased organ but this is at present difficult to arrange with sufficient degree of accuracy. Therefore, the only way seems to be measurement of the working capacity of the body as a whole.

The purpose of the present investigation is to precise more exactly the physiological bases for such determinations and to exemplify the usefulness of a standardized working test for judging patients with various heart and lung diseases.





# PART I. HISTORICAL

## I. Survey of Literature.

The literature on functional tests is very extensive. The following survey covers briefly investigations dealing with tests based on determinations made during and/or after work. The tests may be divided into two groups, (1) maximal tests (tests performed to exhaustion) and (2) submaximal tests.

### 1. Maximal tests.

Hill, Long and Lupton (1924), and Herbst (1928) conclude from standing running experiments, that if a sufficiently large number of muscles are involved, maximum oxygen consumption depends on maximum minute volume of the heart, and should therefore be regarded as a reliable measure of maximum working capacity. Herbst (1928) and Knipping (1937), using an ergometer for arm work, have dealt with such determinations on heart and lung patients, but according to Furusawa (1926) and Hansen (1934) arm work in normal subjects does not involve a sufficient number of muscles. Accepting oxygen intake as a measure of maximum cardiac output is only correct when no lung disease is present. If the lungs are involved, maximum oxygen consumption depends on the capacity of both the circulatory and respiratory systems. For differentiation between pulmonary and circulatory insufficiency Knipping (1934—35) and others working with Knipping's method, have determined the so called oxygen deficiency during rest and work. They conclude that when breathing an air mixture of a higher oxygen percentage than ordinary air, the oxygen intake is greater in lung cases than in heart cases. Knipping's method is however afflicted with technical errors which make the determinations doubtful (Bjerknes 1939, Nager 1947).

Several investigations have been made on the blood lactate level during hard work, but no definite correlation to maximum work-

ing capacity in normal subjects has been established; (Taylor 1944, Johnson and Brouha 1942). Knehr, Dill and Neufeld (1942) found that training for hard work increased the ability to stand high blood lactate levels.

At maximal work,  $p\text{CO}_2$  of expired air is lower than during submaximal work. Briggs (1920) and Schneider (1931) stated that the point where  $p\text{CO}_2$  in expired air begins to sink could be regarded as a sign of the beginning of exhaustion. Taylor (1941) has however shown that this peak in  $p\text{CO}_2$  is most variable, whereas a sudden decrease of alveolar  $p\text{CO}_2$  occurs just before exhaustion.

Taylor (1944) has used a type of maximal test in which the work on a treadmill is rapidly increased until the subject becomes exhausted. The working time to this point is called »the performance index«. He found no significant correlation between this index and maximal heart rate, ventilation or respiratory rate. A small positive correlation was found with maximum oxygen consumption.

According to Knehr, Dill and Neufeld (1942) and Johnson, Brouha and Darling (1942) rate of pulse recovery is found to be unrelated to maximal work.

Maximal work tests are not well suited for clinical use. It is difficult to estimate whether a patient is really working at his utmost, and certain risks are involved in using such tests on non-healthy subjects. Furthermore the ability of sustaining work under anaerobic conditions during short periods, does not necessarily give any information of the capacity of the circulatory and respiratory systems.

## 2. Submaximal tests.

Tests dealing with reactions during work (a), and with reactions after work (b).

a) An ideal method for testing normal subjects and heart patients would be cardiac output determinations. Some investigations of this kind are available.

Means and Newburgh (1915) found no positive difference bet-

ween a patient with compensated valvular disease, and a healthy subject, when tested on a bicycle ergometer with loads up to 600 kg-m/min.

Liljestrand and Zander (1927) using the same kind of work and about the same range of loads, found no abnormal reaction in a case of total heart block. Alt, Walter and Smith (1930) conclude from determinations on 7 patients with chronic rheumatic valvular disease, subacute rheumatic fever or complete heart block, and 4 healthy subjects, that there are no positive differences in cardiac output between the two groups. The test was performed on a bicycle ergometer with 2 loads, 230 and 350 kg-m/min.

There are some other investigations regarding cardiac output, but as pointed out by Nielsen (1935), the number of errors present make the results doubtful. Nielsen determined cardiac output during work on a bicycle ergometer on 13 heart patients with compensated heart disease, and 6 healthy subjects, using work-loads from 335 to 620 kg-m/min. He found that most of the heart patients had a somewhat smaller cardiac output than the normal subjects in relation to oxygen consumption at steady state. Furthermore he found the patients to have a higher pulse rate and ventilation, and a somewhat higher oxygen consumption at the same load of work. However, no statistical treatment of the material was carried out. As to the regularity of increase with work-load of cardiac output, oxygen consumption and ventilation, the heart patients reacted in the same manner as the other subjects.

The oxygen consumption of heart and lung patients has also been investigated by other authors, and has proved to be about the same or somewhat greater than that of healthy subjects during steady state conditions. Meakins and Long (1927), using standing walking (184 steps/min) as test work, found in a few heart patients about the same oxygen consumption as in healthy subjects. Campbell and Sale (1927) investigated 3 heart patients and one healthy subject by having them step on and off a wooden block 13 ins high, for 5—10 minutes. They found oxygen consumption somewhat higher in the heart patients. Campbell (1934), using the same method, arrived at the same conclusion. Eskildsen (1945), on testing 28 heart patients, 19 lung patients and 23 normal

subjects on a bicycle ergometer at 300—500 kg-m/min (some cases at 200—600 kg-m/min), found no differences in the material except when heart and lung patients showed signs of insufficiency during work. These latter subjects had a lower oxygen consumption at the load when signs of insufficiency became apparent.

During work of short duration (1—2 minutes), oxygen consumption of heart and lung patients is often found to be less than for healthy subjects; Peabody and Sturgis (1922, 11 compensated heart patients and 11 normal subjects), Meakins and Long (1927), Herbst (1928, one-arm ergometer), Harrison and Pilcher (1930, 1 compensated heart patient and 3 athletes walking on stairs).

From these results it may be concluded that oxygen consumption in heart and lung patients increases at a slower rate during work than in healthy subjects, but that for a given work it reaches about the same or a slightly higher steady state value.

On the other hand determinations of oxygen debt in relation to total oxygen intake for a given work indicate that some patients may perform work with a lower mechanical efficiency (Campbell and Sale [1927], Harrison and Pilcher [1930]). Katz, Soskin, Schurz, Ackerman and Plaut (1934) found normal values for efficiency at light work.

Eppinger and Hinsberg (1928) found from moderate work of short duration a lower efficiency on some heart patients. Bansi and Groscurth (1930 and 1931) found normal efficiency values for compensated heart patients but lower values for decompensated ones.

According to Peabody and Sturgis (1922), Campbell and Sale (1927), Herbst (1928), Kaltreider and McCann (1937, using a bicycle ergometer), and Eskildsen (1945) compensated heart patients have the same, or a somewhat higher ventilation of the lungs during work than healthy subjects. As a rule, the ventilation is definitely higher in decompensated heart disease [Herbst (1928), Harrison and Pilcher (1930), Eskildsen (1945)]. Herbst (1928) found a higher ventilation in emphysema cases. Patients investigated during asthmatic attacks could not as a rule increase their ventilation above the resting value during work. Campbell (1930) found the increase of ventilation during work in healthy subjects

and heart patients to be about the same, but when considering respiratory rate he states that the heart patients had a diminished alveolar ventilation. This was more pronounced in mitral cases when compared with aortic cases. Kaltreider and McCann (1937), when testing cases with pulmonary fibrosis and emphysema, found higher ventilations during work than in normal cases. Roelsen and Eskildsen (1941) tested silicosis and emphysema cases with asthma and bronchitis at 400 kg-m/min on a bicycle ergometer. They found a definitely higher ventilation in the emphysema cases, a somewhat higher in pronounced silicosis, whereas cases with less pronounced silicosis had normal or slightly elevated ventilation values during work in steady state. Eskildsen (1945) concludes that positive differences between normal subjects and emphysema and asthma patients can only be demonstrated in an advanced state of the disease. Work close to, or at the patients maximum capacity, shows a difference between heart and emphysema-asthma patients. The former having a true hyperventilation, whereas the latter, in spite of pronounced dyspnea, work with relatively low ventilation.

The relation between ventilation and oxygen consumption has been expressed by Herbst (1928), as the amount of oxygen utilized from one liter inspired air. He found lower values with emphysema patients than for others. Knipping and Moncrieff (1932), using the volume of inspired air needed for 100 cc oxygen intake [ventilation equivalent for oxygen, Anthony (1930)], found for 54 healthy subjects a mean value of 2.4 at rest. The complete range of values was however 1.68—3.70. During moderate work the values were unchanged or somewhat lower than at rest. In heart and lung patients, higher values were found at rest and during moderate work in proportion to the degree of failure of the circulatory and respiratory systems. Knipping and Moncrieff state that when no diseases of the circulatory system are present, the »ventilation equivalent for oxygen» is a reliable measure of respiratory capacity.

Respiratory rate in patients during work has not been extensively investigated. Campbell (1934), in an investigation of 9 healthy subjects and 7 heart patients, found for the latter some-

what higher respiratory rates at rest, but definitely higher at light and moderate work. During light work the patients had a mean value of 26 compared to 19 for normal subjects. Some patients could not work adequately at the moderate load. At the light load the average respiratory rate was 32, whereas those who could stand the two tests had at the light load 17 resp/min. Two patients who could only work for a short time at the moderate load had respiratory rates of 40 and 44. Kaltreider and McCann (1937) found at exhausting work on lung and heart patients a mean of 36 resp/min. Eskildsen (1945) gives no figures for respiratory rates, but only for the depth of respiration, which he found diminished in some asthma-emphysema cases.

Lehmann and Michaelis (1941) have proposed a working test which differs from other common types. Pulse rate and pulse amplitude is determined during work on a bicycle ergometer with rapidly increasing load. The working time until the product of these two variables becomes 10,000 is said to be the criterion of working capacity, a longer time indicating a greater capacity. The initial load is, per square centimeter of the calf cross-section, the same for all subjects. The writer has not found any investigation in which this test has been used on heart or lung cases, but Ronge (1948) used it on healthy children for testing their working capacity in a study of the effects of ultra-violet irradiation.

The product of pulse rate and pulse pressure was taken as an approximate estimate of the heart minute volume. It has not however been shown that this product is a real approximation of cardiac output under conditions where no steady state is attained. The test may however be used as an indication of the ability of the body to sustain anaerobic work. Furthermore, it is difficult to understand why the load is adjusted to the calf cross-section.

b) Several tests based on determinations of pulse rate, blood pressure, ventilation, and vital capacity etc., immediately after different types of short-duration work, such as knee bending, walking or running on stairs, or stepping on and off a stool, have not proved to be of value, except in some decompensated cases. In spite of criticism from many authors (among others Britting-

ham and White 1922, Spohr and Lampert 1930, Bang 1932, Hochrein 1938, Heinsen 1942, Kaganas 1943, Bjuggren 1946), they are still widely used.

In consequence of the fact that heart patients may have a greater oxygen debt than healthy subjects, Nylin, in 1931, introduced a cardio-pulmonary functional test, based on determinations of oxygen intake after a short standard work of graded severity on specially constructed stairs. The criterion of working capacity is indicated by the increase of oxygen intake 1—5 minutes after work, as the percentage of the resting value, which ratio he called »relative oxygen debt». Oxygen values are determined by using a Krogh spirometer. Nylin found, in a large number of cases, significant differences between decompensated heart patients and normal subjects. No difference however was found for the compensated patients. Eskildsen (1945), in bicycle ergometer work, came to the same conclusions, using oxygen consumption values for a period of 5 minutes immediately after steady state work. The relative oxygen debt became greater with increasing decompensation. Nylin's method has also been used for lung patients, by Bluhm (1935), and Bruce (1942), who found abnormal values among patients with advanced symptoms. Bruce found that the oxygen values after work were not always reliable. According to change of the pulmonary mid capacity, he sometimes found that the values were too low. From a scatter diagram, Nylin found no relation between body weight and relative oxygen debt, this being accepted as evidence that the test was not influenced by the body weight.

There may however be a correlation but not in the  $x, y$  plane. If the oxygen consumption of the subjects at rest is approximately normal, the metabolism is approximately linearly related to body weight. Therefore relative oxygen debt in relation to weight takes the form  $y = 100 \left( \frac{z}{cx} - 1 \right)$  ( $y$ =relative oxygen debt,  $x$ =body weight,  $c$ =a constant,  $z$ =oxygen intake after work). There may thus be a correlation in a three-dimensional surface, if oxygen consumption after work is not exactly the same function of body weight as at rest, which is unlikely to be the case.

Further discussion on this working test will be found in later pages.



## II. Discussion and criticism of earlier used types of work.

As is apparent from the above survey of literature, different types of physical work have been used in many ways to find the working capacity of an individual, most tests dealing with one or two submaximal loads of work. The following types of work are those mainly employed.

- a) Stepping on and off a step or a stool.
- b) Walking or running on stairs.
- c) " " " " a treadmill.
- d) Standing walking or running.
- e) Arm ergometer work.
- f) Bicycle ergometer work.

One or more of the following functions are used as indicators for determination of working capacity.

- 1) Oxygen consumption.
- 2) Total ventilation.
- 3) The ratio of total ventilation to oxygen consumption.
- 4) Respiratory rate.
- 5) Vital capacity of the lungs.
- 6) Various ratios of ventilation and vital capacity.
- 7) Pulse rate and restitution of pulse rate.
- 8) Oxygen debt.
- 9) Various ratios of pulse rate and blood pressure.
- 10) Time work to exhaustion.

Significant differences between healthy subjects and decompensated patients have usually been shown by different methods, but when testing compensated patients, no, or only slight differences have been found. Testing uncompensated cases is, however, not very important. It is more interesting to estimate the ability of the compensated patients to perform physical work.

Factors that determine or limit working capacity are neuromuscular, circulatory-respiratory and psychological. Tests with determinations of various factors during or after work of short

duration, give a conception of the individual ability of adaptation to work. If such work is stepped up to exhaustion (see Taylor, 1944), one gets a measure of the mental power of the individual and/or the maximal ability of the muscles to work under anaerobic conditions. There is no reason to believe that there should be any simple correlation between the ability of rapid adaptation and the ability of performing a comparatively heavy work during steady state. It seems therefore more important to estimate the highest steady state level. Steady state is a sign of economical working conditions, the reactions of the respiratory and circulatory systems being the most important factors in maintaining equilibrium between oxygen need and oxygen supply. Break down of steady state depends mainly on the inability of maintaining an adequate minute volume of the heart and the inability of the lungs to supply arterial blood with oxygen, provided that a large number of muscles are at work. When small muscle groups are used in a working test, the maximal oxygen consumption attained may not be sufficient for the desired stress on the circulatory and respiratory systems.

In the opinion of the writer, physical working capacity is an individual function. There is probably a continuous decrease of this function from the most well-trained athlete to the heart patient, when decompensated at rest. A completely untrained healthy man may however have the same or a lower working capacity than a trained man with some objective evidence of heart or lung disease.

It may be presumed that the possibility of determining the various working capacities in a group of individuals, depends upon the working intensities employed. One cannot expect to find any greater differences using one or two rather light loads of work. With increased loads the discrepancies ought to be accentuated.

For a test of physical working capacity in the sense suggested, certain conditions are required. A large number of muscles must be involved so that the test will not give a conception only of the working capacity of the muscles. Different and sufficiently heavy loads must be used to make it possible to estimate the maximum steady state level of the subject in question. There must be a

possibility for most of the subjects to attain steady state during moderate loads. The working time must not be too long. A working time of 20—30 minutes or more at several loads, may cause carbohydrate exhaustion and hydrostatic changes of the blood distribution, interfering especially with the circulatory reactions.

The question of the influence of training for the result of a working test has been much discussed. It is not desirable for clinical use to have a test in which some individuals may have a much better mechanical efficiency than others. From experiments in running we know that the efficiency varies considerably in different subjects while running at a given rate (Dill, Talbott and Edwards, 1930; Hill, Long and Lupton, 1924). Oxygen intake in relation to working rate is different in walking and running (Bøje, 1944; Berggren, 1945). These facts show that conclusions arrived at from walking and running experiments, cannot be interpreted in the same way without oxygen determinations. Furthermore, walking in everyday life cannot be considered as training for actual running.

Important conditions for all working tests are standardization and control of the working intensity. It is more or less immaterial if the load is produced by body weight or in any other way, but when comparing different individuals or the same person on different occasions, it must be born in mind that work with body weight as load gives various working intensities when the work is performed in the same manner. For determination of the working intensity, oxygen consumption measurements must be made. If the response of various factors to work are correlated to oxygen consumption, it is possible to make comparisons between individuals.

In most tests employed, body weight is the load, and oxygen measurements are not usually made. Furthermore, working time is often too short for reliable determinations, and the types of work are inconvenient for making various determinations while the work is in progress.

Bicycle ergometer work seems to be superior to almost any type of work for practical use. The following summary shows its advantages.

1. *The bicycle ergometer is a practical apparatus for laboratory work, taking-up small space, and being easy to handle.*
2. *The work can be exactly reproduced.*
3. *A large number of muscles are involved.*
4. *Oxygen consumption is directly related to work-load and the mechanical efficiency determined on various individuals shows comparatively slight differences.*
5. *It is thus possible to make a direct comparison between different subjects and between the reactions at different loads, as there are few extra-movements not taking part in the production of the work output (as compared with e.g. running or walking where a static component is involved).*
6. *Various determinations are easily made during work.*
7. *Working intensity can be adjusted so that the subject is not overloaded.*
8. *Hydrostatic changes of blood distribution may be expected to play a comparatively slight role.*

On the assumption that the subject to be tested has had some opportunity to practise cycling, this type of work may be preferred to any other.

The following factors are considered to be of value in estimating the reaction to work.

*Oxygen consumption* as a measure of the intensity of the work. It can be maximized by heart and lung diseases.

*Ventilation of the lungs* as an indicator of respiratory regulation. Overventilation in relation to oxygen consumption occurring at or near maximal loads, is most easily indicated by increase of the ratio (ventilation per min/oxygen consumption per min).

*Pulse rate* as a factor in cardiac output. The pulse rate has a maximum limit above which there is no increase of cardiac output.

*Respiratory rate* is a limiting factor upon pulmonary ventilation, and an indicator of respiratory regulation.

The other factors mentioned in the summary on page 16 are considered less suitable for determining the response to work. Some of them have already been discussed above. The significance of the vital capacity will be discussed at a later stage.

Only a brief survey of the most important working tests used have been made here. A more detailed description and discussion of some of the tests is found in Cureton's *Physical Fitness Appraisal and Guidance*, 1947.

A short account of the most important observations on the physiological response to physical work under normal conditions, must be given for the understanding of the reaction to work of various functions.

### **III. Some fundamental observations on the physiological response to physical work.**

According to several investigators cardiac output during steady state is a rectilinear function of oxygen consumption, and has been proved for various working intensities up to values corresponding to an oxygen consumption of 2.5—3 liters/min. (Krogh and Lindhard 1912, Lindhard 1914, Boothby 1915, Means and Newburgh 1915, Krogh and Lindhard 1917, Collett and Liljestrand 1924, Bock, Vancaulert, Dill, Fölling and Hurxthal 1928, Hohwü Christensen 1931, Nielsen 1935). Most of these investigations have been based on bicycle ergometer work. Collett and Liljestrand found, that corresponding to a given oxygen consumption, the cardiac output was somewhat lower in better trained subjects. Hohwü Christensen came to the same conclusion, but states that under the same test conditions, the higher cardiac output is found only in completely untrained subjects.

Oxygen consumption during steady state stands in linear relation to the work-load (Lindhard 1914, Boothby 1915, Krogh and Lindhard 1917, Collett and Liljestrand 1924, Liljestrand and Zander 1927, Bock, Vancaulert, Dill, Fölling and Hurxthal 1928, Schneider 1931, Hohwü Christensen 1931/32). Oxygen consumption usually reaches a constant value after 1—2 minutes work, provided the load is not too high (see for instance Liljestrand and Stenström 1920). In very heavy work constant values may not be attained. (According to Hansen, 1934, this is due to increasing

oxygen debt.) Completely untrained subjects have a somewhat higher oxygen consumption at the same work-loads (Hohwü Christensen 1931/32).

Ventilation of the lungs seems to be a rather individual function, but with the same subject it stands in linear relation to oxygen consumption and thus also to work-load (Krogh and Lindhard 1913, Collett and Liljestrand 1924, Schneider 1931, Hohwü Christensen 1931/32, Taylor 1941, Nielsen and Assmusen 1944). Ventilation at a given load has been shown to be lower for better trained subjects than for others (Collett and Liljestrand 1924, Schneider and Crampton 1940, Ebeling and Linxweiler 1940, Bøje 1944).

If oxygen consumption and ventilation are considered as being linear to work-loads, the ventilation per liter oxygen intake (ventilation coefficient for oxygen), is a linear function of work-load. As this line has usually a very small angular coefficient, the ventilation coefficient may be said to be approximately constant. When the load approaches the maximum for the subject, the increase of ventilation is accelerated in relation to oxygen consumption. The ventilation coefficient is thus increasing.

The variability of ventilation between different subjects is reflected in the ventilation coefficient. Lower values have generally been found for better trained subjects (Hohwü Christensen, 1931/32, who found values of approximately 14 to 20).

The increase of respiratory rate with increasing work-load is approximately linear, according to investigations by Schneider (1931), Taylor (1941). When the work begins to exhaust the subject, the increase of respiratory rate may accelerate (Schneider 1931). In very heavy work, close to or at exhaustion, respiratory rates of 30—45 or more have been observed. Sturgis, Peabody, Hall and Fremont-Smith (1922), in 12 subjects, found values between 26 and 45 with an average of 35 resp/min.

Hohwü Christensen (1944) states that the subject has nearly reached his maximum working intensity when the respiratory rate has increased to 30—40 resp/min.

Pulse rate determined in the same time-period of work increases in a linear fashion with increasing loads (Boothby 1915, Krogh

and Lindhard 1917, Collett and Liljestrand 1924, Bock, Vancaulert, Dill, Fölling and Hurxthal 1928, Hohwü Christensen 1931, Schneider 1931, Taylor 1941). When very heavy work leading to exhaustion is considered, pulse rates of approximately 170—200 are found (Lythgoe and Pereira 1915, Gillispie, Gibson and Murray 1925/26, Hohwü Christensen 1931, Knehr, Dill and Neufeld 1942). At higher levels of work the linear relation is not always consistent, pulse rate increase being somewhat retarded in relation to work-load (Bock et al. 1928, Henderson, Haggard and Dolley 1927, Schneider 1931). At a given load the pulse rate in trained subjects is generally less than for others (Henderson et al. 1927, Bock et al. 1928). During training the pulse rate decreases gradually (Hohwü Christensen 1931, Erickson, Simonson, Taylor, Alexander and Keys 1946). Pulse rate at submaximal loads soon reaches a constant value in well-trained subjects (Hohwü Christensen 1931). For men of moderate training the pulse rate seems to increase slightly throughout the working time (Taylor 1941); this increase is more pronounced at heavier loads.

The relation between pulse rate and oxygen consumption or work-load has been treated statistically by a few investigators. Erickson, Simonson, Taylor, Alexander and Keys (1946) found a correlation coefficient of 0.97 between excess oxygen consumption and pulse rate at work on a treadmill; Taylor (1941), with a bicycle ergometer, observed correlations of the same magnitude between work-load and pulse rate. Thus, as previously pointed out by Hohwü Christensen and others, pulse rate in the same subject gives a fairly accurate indication of the severity of the work.

Lundgren (1946) made a thorough investigation of the connection between pulse rate and oxygen consumption, and found a fairly good correspondence between the two variables at different types of lumber work when compared to a bicycle ergometer test.

## PART II. OWN INVESTIGATIONS

### IV. Methods and material.

The following investigation is an attempt to introduce a test for working capacity based on the response to different loads.

The work is performed on a Krogh bicycle ergometer at a pedalling rate of approximately 60 revs/min. The exact velocity of the pedals is indicated by a speedometer attached to the fly-wheel of the bicycle. The men being tested were instructed to keep the speedometer indicator at a certain point, corresponding to a fly-wheel velocity of 5 m/sec. Those who could not keep the indicator steady at this point, were instructed to let it swing the same amount to both sides. Naturally only a minor deviation was allowed. If a man could not be persuaded to work in the above manner, the working intensity could be read from the speedometer provided he worked at a constant rate. In actual fact only a few of the subjects could not perform the test in the correct manner.

The test consisted of an uninterrupted series of work-loads beginning with 300 or 600 kg-m/min, and increasing at approximately every  $6\frac{1}{2}$  minutes by 300 kg-m/min, until the subject could not go on any longer, or until the work at 1200 kg-m/min was accomplished. Some subjects were also tested at higher rates (1500, 1800 etc. kg-m/min).

Pulse rates were determined with a stethoscope at the chest wall for 30 secs, from the beginning of the 3rd, 5th and 7th minute of each load. Ventilation of the lungs and oxygen consumption was determined according to the Douglas bag method for  $2\frac{1}{2}$  minutes from the 4th minute of every work-load. Respiratory rate was determined for one minute beginning some seconds before the 5th minute of every load.



Work-loads and working time was chosen so that most subjects would have a chance of reaching steady state. Some determinations of oxygen consumption and ventilation during a longer working time have proved to give corresponding values between the usual determination (4—6½ min) and one at 8—10½ min (Table 1 A).

TABLE 1 A. *Oxygen consumption, cl/min (a) and ventilation coefficient (b) at 4—6½ (I) and 8—10½ (II) min.*

Case no.	600				900				1200			
	I		II		I		II		I		II	
	a	b	a	b	a	b	a	b	a	b	a	b
15	156	20.3	156	21.0	203	22.6	205	22.8	255	23.5	252	23.3
57	135	25.0	137	24.0	190	23.7	191	24.2	243	22.6	245	23.0
74									247	17.1	246	17.2
80	130	15.1	131	15.1	196	15.6	195	15.8	248	15.9	250	16.3
91									242	19.2	244	19.1
152	149	18.2	150	18.0	199	16.1	199	17.9	251	18.5	250	18.5
361	145	17.9	144	17.8	185	18.4	186	18.6	230	19.3	230	19.3
415	143	16.1	144	16.2	196	16.6	193	16.0	254	18.1	250	18.2
452					179	17.5	180	17.3	236	16.6	236	16.7

Case no.	1500			
	I		II	
	a	b	a	b
361	284	24.9	286	25.0
452	288	17.5	288	17.9

When reproducing the test at short intervals the reactions are about the same. Some differences occur in single functions but the resulting working capacity is not altered (Table 1 B). When the test is repeated several times within a relatively short period training effects may appear sooner or later. It is however not the purpose of this investigation to study the effect of training, consequently only the first test performed is taken into account.

TABLE 1 B. *Pulse rate after 6 min (1), respiratory rate (2), ventilation coefficient (3) and oxygen consumption (4) at tests repeated with a few days interval. I=first test. II=second test.*

Case no.		600				900				1200			
		1	2	3	4	1	2	3	4	1	2	3	4
74	I	114		17.2	148	140		17.8	196	160		17.1	247
	II	110		17.0	150	138		17.3	199	162		17.0	253
91	I	118		18.4	143	148		22.2	198	176		19.2	242
	II	110		18.2	145	144		22.1	200	174		18.9	247
152	I	104		18.2	149	140		19.1	199	176		18.5	251
	II	110		18.0	150	138		19.3	200	174		18.1	249
178	I	124		15.5	143	150		16.4	194	176		19.1	238
	II	118		16.1	145	146		16.4	195	174		19.5	243
187	I	126	18	16.2	122	158	21	16.5	154	188	25	18.4	213
	II	124	16			166	21	16.2	152	194	26	18.4	220
211	I	132	22	19.5	143	164	25	22.6	190	174	30	27.1	235
	II	128	21	19.3	140	160	24	22.2	191	176	29	26.7	238
361	I	106		17.9	145	124		18.4	185	144		19.3	230
	II	96		18.3	138	118		18.4	181	142		19.0	234
451	I	112	15			126	17			148	20		
	II	108	15			128	18			146	22		

Case no.		1500				1800	
		1	2	3	4	1	2
361	I	168		24.9	284		
	II	166		23.8	287		
451	I	161	25			178	30
	II	164	25			174	31

In the statistical treatment of the material, current methods are employed.

#### Key to signs used.

$n$ =number of variates, number of values.

$M$ =arithmetical mean.

$s$ =standard deviation, calculated with respect to the corresponding degree of freedom.

$m$ =standard error of  $M = \frac{s}{\sqrt{n}}$

$V$ =coefficient of variation  $= \frac{s}{M} \cdot 100$

$D$ =difference between two means.

$d$ =standard error of  $D = \sqrt{m_1^2 + m_2^2}$

$r$ =coefficient of correlation. Standard error of  $r = \frac{1-r^2}{\sqrt{n}}$

The significance of differences between mean values are analysed according to Student's  $t$ -test on the assumption that the two means are from the same normal population.

In analysis of variances according to Fisher and Snedecor, the variance ratio is denoted by  $F$ .

One, two, and three asterisks, denote significance of  $t$  and  $F$  values at the 5%, 1% and 0.1% level respectively. The corresponding degrees of significance are said to be probable, significant, and highly significant.

Out of a total of 469 men tested, 189 are work-men at an ore smelting works, most of whom complained of respiratory troubles at work. They have all been medically examined at the Medical Clinic of the Karolinska hospital. Among these men are a few who had had no symptoms, and were found to have no abnormal signs at the medical examination. They have been transferred to the group of healthy subjects in this investigation. This group consists of 40 cases, all of which have been tested in the usual manner. 26 healthy subjects, only tested on their pulse reaction to work at 600—1500 kg-m/min, constitute another normal group.

They are military recruits who had completed some months of their training, and are regarded as a group between the normal healthy subjects and a group of 27 athletes. Most of the latter were medically examined at the Health Control Station of the Swedish Athletic Association.

Of the remaining 188 cases, the majority were men on military service complaining of heart or respiratory troubles at work. Some cases have been medically examined at the Garrison Hospital in Stockholm, but most of them at the out-patient department of the Karolinska hospital.

Medical examination included case history, auscultation of the heart, blood-pressure, x-ray examination of the heart<sup>1)</sup> and lungs, sedimentation rate of the blood and urine analysis for protein and sugar.

At the Laboratory of Clinical Physiology the following determinations were made. Body height and weight, vital capacity of the lungs and electrocardiogram at rest. In all cases an electrocardiogram was taken immediately after work, and additionally in most cases after an elapse of 3 minutes. A spirometric examination of lung volume and its subdivisions (Birath, 1944) was also made at the laboratory on the work-men from the ore smelting works.

All pulse and respiratory rates, ventilations and most of the vital capacity and gas analyses were made by the writer himself. With the exception of some of the athletes the tests were performed before noon, most subjects having had a light meal more than 3 hours before. The subjects had to rest in a recumbent position at least half an hour before the test.

In order to get a general impression of how the objective symptoms were distributed in a material complaining of circulatory and respiratory troubles, no special selection of the military cases was made. For convenience the material is divided into groups according to diagnoses, or to objective symptoms if no positive diagnosis could be made. Thus the following groups have been established.

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<sup>1)</sup> Heart volumes determined according to Jonsell—Rohrer—Kahlstorf.

	Number of cases	Age	
		Mean	Range
1. Rheumatic valvular disease of the heart	16	27	(21—44)
2. Congenital diseases of the heart . . . . .	11	27	(21—42)
3. Suspected congenital diseases of the heart	8	25	(20—35)
4. Suspected rheumatic valvular diseases of the heart . . . . .	23	27	(21—40)
5. Hypertension . . . . .	25	37	(21—57)
6. Actual or suspected acut myocarditis ..	27	26	(19—47)
7. Enlarged hearts . . . . .	29	35	(20—62)
8. Various electrocardiographic abnormal- lities . . . . .	29	30	(20—47)
9. No objective signs of heart or lung disease . . . . .	68	27	(21—44)
10. Emphysema of the lungs and signs of heart disease . . . . .	37	50	(30—66)
11. Suspected emphysema of the lungs ..	16	50	(32—66)
12. Emphysema of the lungs . . . . .	20	48	(39—61)
13. Tracheo-bronchitis without signs of emphysema . . . . .	67	40	(26—58)
14. Ordinary healthy subjects . . . . .	40	32	(20—60)
15. Moderately trained healthy subjects ..	26	21	(21—23)
16. Athletes . . . . .	27	27	(19—43)

Group 5 contains patients with blood pressure above 155 systolic or 100 mm Hg diastolic. Heart volumes larger than 450 cc per square meter body surface are considered to be above normal. Suspected emphysema means a residual air/total lung volume ratio from 30 to 33 %/. Cases with values 33 %/, or more, are said to have emphysema. In group 10 cases with more than 30 %/ and with signs of hypertension, enlarged hearts or coronary insufficiency are included. A more detailed description of the findings in each group follows in later pages in connection with the results of the working test.

## V. Results of determinations of oxygen consumption, pulse rate and respiratory functions at work.

### 1. Oxygen consumption.<sup>1)</sup>

As indicated by Table 1 A, oxygen consumption at the various loads has reached a steady value at the usual time used for the determination in this study. The differences between the loads are seen to be about equal. In some of the cases belonging to group 1, there are however lesser increases from 900 to 1200 kg-m/min, compared to 600—900 kg-m/min. This shows that these patients are at the absolute limit of their working capacity, but as these

<sup>1)</sup> In the following oxygen consumption stands for excess oxygen consumption.

TABLE 2 A. *Excess oxygen consumption (liters/min). Cases with determinations at each of the three loads.*

Group	n	600		900		1200	
		M $\pm$ m	V	M $\pm$ m	V	M $\pm$ m	V
1	12	1.38 $\pm$ 0.050	12.3	1.90 $\pm$ 0.046	8.4	2.38 $\pm$ 0.089	12.9
2	7	1.41 $\pm$ 0.037	8.0	1.91 $\pm$ 0.065	9.9	2.48 $\pm$ 0.059	9.3
3	8	1.36 $\pm$ 0.037	7.5	1.85 $\pm$ 0.034	5.8	2.35 $\pm$ 0.063	7.8
4	18	1.42 $\pm$ 0.030	8.1	1.87 $\pm$ 0.043	8.7	2.42 $\pm$ 0.060	9.5
5	19	1.40 $\pm$ 0.033	10.1	1.95 $\pm$ 0.028	6.0	2.47 $\pm$ 0.037	6.7
6	24	1.41 $\pm$ 0.018	6.4	1.92 $\pm$ 0.027	6.4	2.43 $\pm$ 0.033	6.3
7	24	1.42 $\pm$ 0.021	8.6	1.92 $\pm$ 0.019	5.0	2.45 $\pm$ 0.025	5.1
8	19	1.32 $\pm$ 0.035	11.2	1.85 $\pm$ 0.010	9.1	2.36 $\pm$ 0.059	10.5
9	53	1.38 $\pm$ 0.022	7.8	1.87 $\pm$ 0.021	8.0	2.37 $\pm$ 0.031	9.3
10	22	1.41 $\pm$ 0.036	9.9	1.87 $\pm$ 0.035	7.8	2.38 $\pm$ 0.038	6.2
11	8	1.29 $\pm$ 0.037	8.8	1.83 $\pm$ 0.067	11.0	2.33 $\pm$ 0.098	12.3
12	11	1.35 $\pm$ 0.025	7.7	1.81 $\pm$ 0.038	8.5	2.29 $\pm$ 0.041	5.9
13	47	1.35 $\pm$ 0.015	7.8	1.87 $\pm$ 0.017	7.0	2.38 $\pm$ 0.022	7.2
14	27	1.36 $\pm$ 0.024	9.3	1.87 $\pm$ 0.018	4.7	2.39 $\pm$ 0.022	5.3
16	10	1.47 $\pm$ 0.062	10.9	1.94 $\pm$ 0.052	7.2	2.40 $\pm$ 0.019	5.4
Tot.	309	1.38 $\pm$ 0.007	8.9	1.88 $\pm$ 0.008	7.5	2.39 $\pm$ 0.011	7.6

cases are rather few they do not influence the averages in Tables 2 to any extent.

It can be concluded from Table 2 A that the increase of oxygen consumption at 900—1200 kg-m/min is generally equal to the increase at 600—900 kg-m/min; and that the variability in the groups is fairly uniform. The results as a whole are not altered when comparing the oxygen consumption averages that include all determinations at the different loads (Table 2 B). A closer examination of the variability (Table 3 A and B) at the three loads, shows that there may be some slight differences between the groups, but the differences between the group-averages are of the same magnitude at the various loads.

Another way of showing the steady state conditions in a working test of this kind, consists of determination of mechanical

TABLE 2 B. *Excess oxygen consumption (liters/min). All determinations included.*

Group	600			900			1200		
	n	M $\pm$ m	V	n	M $\pm$ m	V	n	M $\pm$ m	V
1	16	1.40 $\pm$ 0.042	12.1	15	1.87 $\pm$ 0.038	7.8	13	2.38 $\pm$ 0.089	12.9
2	10	1.38 $\pm$ 0.035	7.9	7	1.91 $\pm$ 0.054	9.0	8	2.43 $\pm$ 0.059	9.3
3	8	1.37 $\pm$ 0.037	7.5	8	1.85 $\pm$ 0.034	5.8	7	2.35 $\pm$ 0.053	7.8
4	23	1.42 $\pm$ 0.027	9.7	20	1.87 $\pm$ 0.043	8.7	20	2.41 $\pm$ 0.053	6.8
5	23	1.41 $\pm$ 0.029	9.6	23	1.96 $\pm$ 0.027	6.4	20	2.47 $\pm$ 0.037	6.8
6	27	1.41 $\pm$ 0.019	6.9	26	1.92 $\pm$ 0.024	6.2	24	2.43 $\pm$ 0.033	6.8
7	25	1.42 $\pm$ 0.024	8.6	27	1.93 $\pm$ 0.020	5.2	26	2.46 $\pm$ 0.025	5.2
8	24	1.34 $\pm$ 0.038	13.1	21	1.86 $\pm$ 0.040	9.3	20	2.36 $\pm$ 0.059	10.5
9	65	1.38 $\pm$ 0.012	7.2	65	1.87 $\pm$ 0.017	6.9	57	2.37 $\pm$ 0.029	9.2
10	29	1.41 $\pm$ 0.029	9.2	27	1.86 $\pm$ 0.034	7.6	23	2.38 $\pm$ 0.038	6.2
11	10	1.32 $\pm$ 0.039	9.3	13	1.84 $\pm$ 0.045	9.0	10	2.33 $\pm$ 0.079	11.1
12	17	1.28 $\pm$ 0.029	8.1	13	1.78 $\pm$ 0.037	9.5	12	2.26 $\pm$ 0.035	6.5
13	61	1.34 $\pm$ 0.013	8.1	56	1.87 $\pm$ 0.019	7.9	54	2.38 $\pm$ 0.021	6.9
14	30	1.36 $\pm$ 0.021	9.0	29	1.88 $\pm$ 0.015	4.3	30	2.38 $\pm$ 0.022	5.3
16	11	1.47 $\pm$ 0.053	10.1	19	1.91 $\pm$ 0.032	7.2	20	2.40 $\pm$ 0.029	5.1
Tot.	379	1.38 $\pm$ 0.006	8.9	372	1.88 $\pm$ 0.007	7.5	344	2.39 $\pm$ 0.010	7.8

TABLE 3. *Analysis of variance of oxygen consumption between and within groups.*

*A. Corresponding to Table 2 A.*

*B. Corresponding to Table 2 B.*

	Load	Degrees of freedom		F
		1	2	
A	600	14	294	1.72
	900	14	294	1.44
	1200	14	294	1.40
B	600	14	364	*2.06
	900	14	357	*2.07
	1200	14	329	1.66

TABLE 4 A. *Mechanical efficiency percentages.*  
*Corresponding to Table 2 A.*

Group	M $\pm$ m		
	600	900	1200
1	20.7 $\pm$ 0.44	22.5 $\pm$ 0.55	24.1 $\pm$ 0.90
2	20.3 $\pm$ 0.58	22.5 $\pm$ 0.79	23.5 $\pm$ 0.77
3	20.9 $\pm$ 0.52	23.4 $\pm$ 0.48	24.8 $\pm$ 0.69
4	20.2 $\pm$ 0.42	23.0 $\pm$ 0.52	23.7 $\pm$ 0.58
5	20.5 $\pm$ 0.47	22.1 $\pm$ 0.30	23.2 $\pm$ 0.36
6	20.4 $\pm$ 0.27	22.4 $\pm$ 0.30	23.6 $\pm$ 0.31
7	20.3 $\pm$ 0.35	22.3 $\pm$ 0.23	23.4 $\pm$ 0.24
8	21.7 $\pm$ 0.57	23.3 $\pm$ 0.50	24.3 $\pm$ 0.60
9	20.4 $\pm$ 0.22	22.6 $\pm$ 0.25	24.1 $\pm$ 0.32
10	20.4 $\pm$ 0.50	23.0 $\pm$ 0.45	24.1 $\pm$ 0.33
11	22.3 $\pm$ 0.69	24.1 $\pm$ 0.93	25.5 $\pm$ 1.11
12	21.4 $\pm$ 0.41	23.7 $\pm$ 0.51	25.1 $\pm$ 0.37
13	21.6 $\pm$ 0.22	23.4 $\pm$ 0.22	24.1 $\pm$ 0.23
14	21.1 $\pm$ 0.36	23.0 $\pm$ 0.20	24.0 $\pm$ 0.24
16	19.5 $\pm$ 0.80	22.1 $\pm$ 0.61	23.9 $\pm$ 0.49
Tot.	20.8 $\pm$ 0.09	22.9 $\pm$ 0.09	24.0 $\pm$ 0.10



efficiency. Assuming that 1 liter of oxygen corresponds to 4.9 Cal. the net efficiencies will be shown in Tables 4. In general, the values are the same as found by other investigators for this type of work.

TABLE 4 B. *Mechanical efficiency percentages.*  
Corresponding to Table 2 B.

Group	M $\pm$ m		
	600	900	1200
1	20.5 $\pm$ 0.61	23.0 $\pm$ 0.47	24.1 $\pm$ 0.89
2	20.6 $\pm$ 0.51	22.6 $\pm$ 0.64	23.5 $\pm$ 0.77
3	20.9 $\pm$ 0.52	23.4 $\pm$ 0.47	24.8 $\pm$ 0.69
4	20.1 $\pm$ 0.48	23.0 $\pm$ 0.64	23.8 $\pm$ 0.52
5	20.3 $\pm$ 0.42	21.0 $\pm$ 0.30	23.3 $\pm$ 0.35
6	20.3 $\pm$ 0.28	22.4 $\pm$ 0.28	23.6 $\pm$ 0.31
7	20.3 $\pm$ 0.34	22.3 $\pm$ 0.23	23.3 $\pm$ 0.24
8	21.4 $\pm$ 0.61	23.1 $\pm$ 0.50	24.3 $\pm$ 0.60
9	20.8 $\pm$ 0.19	23.0 $\pm$ 0.20	24.2 $\pm$ 0.30
10	20.3 $\pm$ 0.55	23.1 $\pm$ 0.43	24.1 $\pm$ 0.33
11	21.7 $\pm$ 0.64	23.7 $\pm$ 0.59	25.4 $\pm$ 0.89
12	21.6 $\pm$ 0.35	24.3 $\pm$ 0.50	25.1 $\pm$ 0.38
13	21.3 $\pm$ 0.21	22.0 $\pm$ 0.23	24.1 $\pm$ 0.21
14	21.1 $\pm$ 0.33	22.9 $\pm$ 0.18	24.1 $\pm$ 0.23
16	21.4 $\pm$ 0.69	23.5 $\pm$ 0.37	24.4 $\pm$ 0.28
Tot.	20.8 $\pm$ 0.09	22.9 $\pm$ 0.09	24.0 $\pm$ 0.10

We may conclude that provided the person to be tested is not at the point of exhaustion, it is possible to get an estimate of oxygen consumption at the different loads without making a special determination. In the majority of instances all working tests of interest are with such cases. Oxygen consumption is indirectly estimated from work-load within a range of  $\pm 8\%$  in  $\frac{2}{3}$  of the cases. However, for a more accurate determination, oxygen consumption must always be measured directly.

## 2. Pulse rate.

The average pulse levels after 6 minutes work at 600, 900 and 1200 kg-m/min have been calculated for each group. Table 5 A shows the values for those cases which have worked at all three loads. Table 5 B gives the average pulse rates for all cases. An analysis of variance is summed up in Table 6.

TABLE 5 A. *Pulse rates.*  
*Cases with determinations at each of the three loads.*

Group	n	M $\pm$ m		
		600	900	1200
1	13	128.5 $\pm$ 3.1	154.8 $\pm$ 3.1	179.1 $\pm$ 2.6
2	9	123.8 $\pm$ 3.2	150.2 $\pm$ 4.0	171.8 $\pm$ 5.0
3	7	113.7 $\pm$ 5.8	138.0 $\pm$ 5.1	161.7 $\pm$ 3.9
4	21	118.6 $\pm$ 2.9	141.7 $\pm$ 3.6	162.7 $\pm$ 3.1
5	21	121.3 $\pm$ 4.1	144.6 $\pm$ 4.0	167.2 $\pm$ 3.8
6	24	125.3 $\pm$ 4.6	152.0 $\pm$ 4.0	175.1 $\pm$ 4.1
7	26	109.3 $\pm$ 2.9	133.9 $\pm$ 4.0	159.3 $\pm$ 3.0
8	26	117.5 $\pm$ 3.3	140.7 $\pm$ 3.9	163.2 $\pm$ 3.7
9	59	124.7 $\pm$ 1.8	148.8 $\pm$ 2.0	169.6 $\pm$ 1.9
10	24	111.8 $\pm$ 3.1	132.6 $\pm$ 3.4	156.7 $\pm$ 3.8
11	11	110.7 $\pm$ 3.3	136.2 $\pm$ 3.3	156.4 $\pm$ 2.8
12	11	106.4 $\pm$ 2.6	130.9 $\pm$ 3.6	151.8 $\pm$ 3.4
13	57	109.8 $\pm$ 2.1	137.5 $\pm$ 1.8	160.1 $\pm$ 1.6
14	40	115.3 $\pm$ 2.2	137.4 $\pm$ 2.2	158.5 $\pm$ 1.7
15	26	109.5 $\pm$ 2.1	125.5 $\pm$ 2.1	142.5 $\pm$ 2.7
16	21	99.9 $\pm$ 2.7	117.4 $\pm$ 2.9	136.4 $\pm$ 3.8

As indicated by the F-values in Table 6, there are highly significant differences between the groups at all the three loads; it is obvious that these differences are increased according to the load.

It is interesting to note the differences between group 14 (normal subjects) and the other groups. In Tables 7 the t-values for these differences are tabulated for each load. Those heart cases that are least capable of doing work, as judged by the objective

TABLE 5 B. *Pulse rates.*  
*All values included.*

Group	600		900		1200	
	n	M $\pm$ m	n	M $\pm$ m	n	M $\pm$ m
1	16	131.5 $\pm$ 6.0	16	157.5 $\pm$ 3.5	13	179.1 $\pm$ 2.6
2	11	126.2 $\pm$ 4.3	10	150.2 $\pm$ 3.6	9	171.8 $\pm$ 5.0
3	8	119.0 $\pm$ 5.8	8	142.0 $\pm$ 5.1	7	161.7 $\pm$ 3.9
4	23	118.0 $\pm$ 2.4	23	140.6 $\pm$ 3.4	21	162.7 $\pm$ 3.1
5	25	124.1 $\pm$ 4.1	25	148.1 $\pm$ 4.2	21	167.2 $\pm$ 3.8
6	27	127.9 $\pm$ 4.5	26	154.2 $\pm$ 3.9	24	175.1 $\pm$ 4.1
7	28	109.6 $\pm$ 2.8	29	133.9 $\pm$ 3.8	26	159.3 $\pm$ 3.0
8	29	117.5 $\pm$ 2.2	27	140.7 $\pm$ 4.1	26	163.2 $\pm$ 3.7
9	68	126.5 $\pm$ 1.8	68	150.1 $\pm$ 1.9	69	169.6 $\pm$ 1.9
10	31	118.0 $\pm$ 3.7	29	134.7 $\pm$ 3.6	24	156.7 $\pm$ 3.3
11	14	111.3 $\pm$ 3.1	14	137.4 $\pm$ 3.3	11	156.4 $\pm$ 2.8
12	18	109.1 $\pm$ 2.3	14	133.9 $\pm$ 3.3	12	153.8 $\pm$ 3.4
13	67	110.0 $\pm$ 1.5	65	135.4 $\pm$ 1.8	68	160.1 $\pm$ 1.6
16	21	99.9 $\pm$ 2.7	27	117.6 $\pm$ 2.7	27	137.3 $\pm$ 3.4

TABLE 6. *Analysis of variance of pulse rates between and within groups.*

A. Corresponding to Table 5 A.

B. Corresponding to Table 5 B.

	Load	Degrees of freedom		F
		1	2	
A	600	16	380	***6.77
	900	16	380	***9.00
	1200	16	380	***12.13
B	600	15	436	***8.46
	900	15	431	***10.88
	1200	15	388	***12.72

and subjective symptoms, belong to groups 1 and 6. The differences for these groups are seen to increase with the load. With group 1 it is already significant at 600 kg-m/min, whereas with group 6 it does not occur before 900 kg-m/min. Other groups containing heart patients (groups 2, 5) show probable or nearly significant discrepancies in pulse rates when compared to group 14, while lung cases (groups 10, 11, 12) show no differences at all. In the case of group 9 significant differences are seen at each of the three loads.

TABLE 7 A. *Differences of pulse rate means between the normal and other groups.*

*Corresponding to Table 5 A.*

Group	f	600		900		1200	
		D	t	D	t	D	t
1	51	13.2	*3.16	17.4	***4.04	20.6	***6.10
2	47	8.5	1.77	12.9	*2.52	18.3	*3.06
3	45	— 1.6	0.60	0.6	0.83	3.2	1.30
4	59	3.3	0.90	4.4	1.04	1.3	0.36
5	59	5.9	1.22	7.3	1.73	8.7	*2.38
6	62	10.0	*2.20	14.7	*3.46	16.6	***4.31
7	64	— 6.0	1.66	— 3.4	0.75	0.8	0.37
8	64	2.2	0.55	3.3	0.78	4.8	1.33
9	97	9.4	***3.35	11.2	***3.70	11.1	***4.02
10	62	— 3.5	0.81	— 4.8	1.20	— 1.7	0.45
11	49	— 4.4	0.75	— 1.2	0.32	— 2.1	0.28
12	49	— 8.9	1.98	— 6.6	1.48	— 6.7	1.80
13	95	— 5.5	1.58	0.2	0.05	1.7	0.73
15	64	— 5.8	1.83	— 11.8	***3.80	— 16.0	***5.24
16	59	— 15.4	***4.38	— 19.9	***5.37	— 22.1	***6.08

The hypernormal cases in groups 15 and 16 differ significantly from group 14 except at 600 kg-m/min in the case of group 15.

On the assumption that pulse rate is a linear function of workload, the angular coefficients (b) for these variables are calculated according to the method of least squares. For convenience the

TABLE 7 B. Differences of pulse rate means between the normal and other groups.

Corresponding to Table 5 B.

Group	600			900			1200		
	f	D	t	f	D	t	f	D	t
1	54	16.2	**3.20	54	20.2	***4.88	51	20.6	***6.10
2	49	10.9	*2.22	48	12.9	*2.55	47	13.3	**3.06
3	46	3.7	0.60	46	4.7	0.83	45	3.2	1.30
4	61	2.7	0.75	61	3.3	0.75	59	1.3	0.36
5	63	8.8	1.71	63	10.7	*2.43	59	8.5	*2.38
6	65	12.6	**2.84	64	15.5	***3.77	62	16.6	***4.31
7	66	-5.7	1.63	67	-3.5	0.78	64	0.8	0.37
8	67	2.2	0.57	65	3.4	1.70	64	4.7	1.33
9	106	11.2	***3.97	106	12.8	***3.31	97	12.7	***4.02
10	69	2.7	0.62	67	-2.6	0.62	62	0.5	0.45
11	53	-4.0	0.87	52	0.1	0.02	49	-2.1	0.28
12	56	-6.2	1.81	52	-3.5	0.88	50	-4.7	1.00
13	105	-5.3	1.91	103	-2.0	0.69	95	1.7	0.73
16	59	-15.4	***4.38	65	-19.7	***5.61	65	-21.1	***5.58

TABLE 8. Angular coefficients based on pulse rate and work load divided by 300.

Group	M	s	n	Group	M	s	n
1	25.3	4.9	13	9	24.2	6.2	59
2	24.0'	2.1	9	10	23.6	5.6	24
3	22.5	5.2	7	11	21.8	4.9	11
4	20.6	3.8	21	12	21.9	4.7	11
5	23.9	5.4	21	13	22.4	5.4	57
6	24.3	5.9	24	14	21.4	4.7	40
7	25.1	5.0	26	15	16.5	4.3	26
8	23.1	4.6	26	16	17.3	4.3	21

calculations are based on work-loads divided by 100. The values of 3b are given in Table 8. The high values of b appear to be related to high pulse rates at 1200 kg-m/min. An analysis of

variance of the differences between the group-averages of 3b gives  $F=6.60^{***}$  for 15 and 380 degrees of freedom and is thus highly significant.

As mentioned in the survey of literature the linear relation of pulse rate to oxygen consumption or work-load has not always been found correct at heavier loads. For this reason and because most workers in this field have largely used selected subjects, it is interesting to investigate this problem in a comprehensive, mixed material.

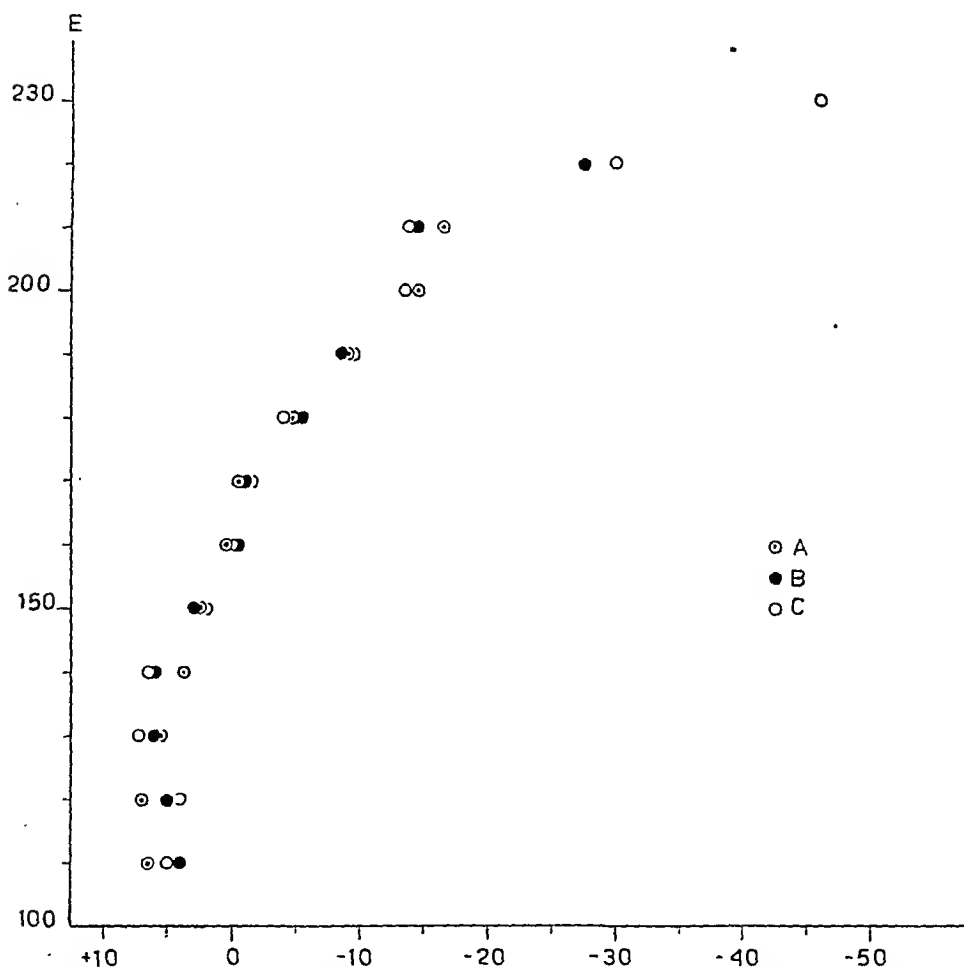


Fig. 1. Mean values of *D* for classes of *E*. A calculations based on work-loads, B on oxygen consumptions and C on work-loads for cases included in B. Vertical axis: estimated pulse rates. Horizontal axis: difference between observed and estimated pulse rates.

The mean values of pulse rates in Table 5 A show only a slightly smaller increase in pulse rates from 900—1200 kg-m/min compared to 600—900 kg-m/min for groups with the highest pulse levels. This does not definitely disagree with the linear relationship.

For a closer examination the writer has however adopted another procedure. The pulse values at 1200 kg-m/min have been estimated from the values at 600 and 900 kg-m/min on the assumption that pulse rate is a linear function of work-load. These estimates are compared with the observed values at 1200 kg-m/min. The difference, observed value minus estimated value ( $=D$ ), and the corresponding estimated value ( $E$ ) for each case, was represented in a scatter diagram.

In Fig. 1 are shown the averages of  $D$  for each class interval of 10 beats/min of  $E$ . If the pulse rates at the three loads are linearly related, the averages for each class of  $E$  should not differ from zero. In Table 9 the class averages of  $D$  are calculated together with  $t$ -values for discrepancies. In general, a linear relation exists when  $E$  falls between 155 and 175 beats/min. Below this range the pulse curves are inclined, whereas above they are declined. Table 9 explains why no positive conclusions as to the linearity of the pulse curves can be drawn from Table 5 A, in

TABLE 9. Means of differences ( $D$ ) between observed and estimated pulse rates at 1200 kg-m/min for classes of estimated pulse rates ( $E$ ), based on work-loads.  $t$ -values indicate degree of significance for departure of  $D$  from zero.

E	D	s	n	t	E	D	s	n	t
110	6.3	3.2	12	***6.81	180	- 4.7	5.6	45	***5.82
120	6.8	9.7	10	2.22	190	- 8.8	6.2	30	***7.95
130	5.3	6.4	25	***4.75	200	- 14.4	7.8	22	***8.65
140	3.8	7.9	48	***3.61	210	- 16.4	6.4	11	***8.44
150	2.5	6.4	63	**3.14	220	- 30		1	
160	0.4	5.9	67	0.59	230	- 46		1	
170	- 0.6	7.2	61	0.64					

TABLE 10. Differences between observed and estimated pulse rates at 1200 kg-m/min for classes of estimated pulse rates, based on oxygen consumptions. *t*-values as in Table 9.

E	D	s	n	t	E	D	s	n	t
110	4.0	2.0	3	*3.48	170	— 0.9	6.6	58	1.07
120	5.0	9.6	4	1.04	180	— 5.6	9.1	40	***4.04
130	5.8	6.9	17	**3.45	190	— 8.1	6.3	19	***5.40
140	6.0	7.8	30	***4.34	200	— 13.4	7.0	18	***8.48
150	2.7	5.8	47	**3.32	210	— 14.7	4.9	9	***8.99
160	— 0.3	7.1	56	0.37	220	— 27.3	7.3	8	***10.52

TABLE 11. A. Differences between observed and estimated pulse rates at 1200 kg-m/min based on work-load, for all cases represented in Table 10.

B. Differences between means calculated according to either method.

E	A.				B.
	D	s	n	t	$D_1 \pm d$
110	5.0	4.5	2	1.58	$1.0 \pm 3.4$
120	4.0	7.9	5	1.14	$1.0 \pm 5.9$
130	7.2	5.8	22	***5.78	$1.4 \pm 2.1$
140	6.6	7.5	33	***5.04	$0.6 \pm 1.9$
150	2.4	6.5	52	*2.78	$0.3 \pm 1.2$
160	0.0	6.0	57	0.05	$0.4 \pm 1.3$
170	— 1.2	7.3	53	1.17	$0.3 \pm 1.3$
180	— 4.0	5.5	40	***4.54	$1.7 \pm 1.6$
190	— 9.4	6.4	22	***6.90	$1.3 \pm 2.0$
200	— 13.4	5.8	13	***8.26	$0.0 \pm 2.3$
210	— 13.8	4.3	8	***8.99	$0.9 \pm 2.2$
220	— 30		1		
230	— 46		1		



which the average pulse rates consist of values from different pulse levels.

It may be concluded that the shape of the pulse curve is influenced by the pulse level.

For completeness and partly for control of the relation of the oxygen consumption to work-load, the same procedure has been repeated with oxygen consumption values (Table 10, Fig. 1). Table 11 A corresponds to Table 9, and Table 11 B shows that generally speaking no differences exist in using work-load instead of oxygen consumption.

### 3. Ventilation of the lungs.

For convenience, ventilation is expressed as ventilation per liter oxygen consumption. Under ideal conditions this quotient (ventilation coefficient for oxygen) increases slightly with the load.

TABLE 12 A. *Ventilation coefficients.*  
*Cases with values at each of the three loads.*

Group	n	$M \pm m$		
		600	900	1200
1	12	$19.7 \pm 0.79$	$21.2 \pm 0.93$	$25.0 \pm 1.12$
2	8	$17.6 \pm 1.01$	$19.0 \pm 1.00$	$21.1 \pm 1.17$
3	7	$18.4 \pm 0.48$	$18.3 \pm 0.52$	$19.9 \pm 0.67$
4	18	$19.6 \pm 0.76$	$19.9 \pm 0.62$	$20.8 \pm 0.60$
5	19	$17.9 \pm 0.65$	$18.7 \pm 0.70$	$19.5 \pm 0.90$
6	24	$18.9 \pm 0.53$	$19.5 \pm 0.61$	$20.6 \pm 0.58$
7	24	$18.0 \pm 0.40$	$18.6 \pm 0.47$	$20.0 \pm 0.66$
8	19	$19.1 \pm 0.48$	$20.5 \pm 0.54$	$22.6 \pm 1.10$
9	53	$19.8 \pm 0.48$	$20.1 \pm 0.44$	$21.5 \pm 0.58$
10	22	$19.6 \pm 0.65$	$20.5 \pm 0.76$	$22.3 \pm 0.88$
11	8	$17.4 \pm 0.75$	$17.8 \pm 0.62$	$19.4 \pm 0.82$
12	11	$18.8 \pm 0.67$	$19.9 \pm 0.80$	$21.8 \pm 0.90$
13	47	$17.8 \pm 0.26$	$18.3 \pm 0.31$	$20.3 \pm 0.38$
14	27	$17.6 \pm 0.54$	$17.9 \pm 0.43$	$19.4 \pm 0.47$
16	10	$16.3 \pm 0.40$	$16.5 \pm 0.50$	$17.0 \pm 0.71$

TABLE 12 B. *Ventilation coefficients.*  
*All values included.*

Group	600		900		1200	
	n	M $\pm$ m	n	M $\pm$ m	n	M $\pm$ m
1	16	20.4 $\pm$ 0.87	15	22.3 $\pm$ 0.87	13	25.0 $\pm$ 1.12
2	10	20.6 $\pm$ 2.22	10	20.3 $\pm$ 1.58	8	21.1 $\pm$ 1.17
3	8	18.5 $\pm$ 0.42	8	18.6 $\pm$ 0.56	7	19.9 $\pm$ 0.67
4	23	19.2 $\pm$ 0.73	20	20.0 $\pm$ 0.62	20	20.4 $\pm$ 0.60
5	23	18.6 $\pm$ 0.69	23	19.6 $\pm$ 0.84	20	19.9 $\pm$ 0.86
6	27	19.5 $\pm$ 0.52	26	19.8 $\pm$ 0.61	24	20.6 $\pm$ 0.58
7	25	18.0 $\pm$ 0.40	27	18.7 $\pm$ 0.46	26	20.2 $\pm$ 0.62
8	24	19.3 $\pm$ 0.61	21	20.8 $\pm$ 0.61	20	22.5 $\pm$ 1.10
9	65	19.9 $\pm$ 0.51	65	20.5 $\pm$ 0.39	57	21.6 $\pm$ 0.54
10	29	21.0 $\pm$ 0.72	27	20.8 $\pm$ 0.71	23	22.3 $\pm$ 0.88
11	10	17.5 $\pm$ 0.56	13	17.6 $\pm$ 0.52	10	19.1 $\pm$ 0.64
12	17	18.6 $\pm$ 0.54	13	19.9 $\pm$ 0.68	12	21.8 $\pm$ 0.84
13	61	17.7 $\pm$ 0.16	56	18.4 $\pm$ 0.31	54	20.1 $\pm$ 0.36
14	30	17.6 $\pm$ 0.53	29	18.1 $\pm$ 0.88	30	19.5 $\pm$ 0.44
16	11	16.3 $\pm$ 0.40	19	16.6 $\pm$ 0.31	20	17.0 $\pm$ 0.71

TABLE 13. *Analysis of variance of ventilation coefficients between and within groups.*

*A. Corresponding to Table 12 A.*

*B. Corresponding to Table 12 B.*

Load		Degrees of freedom		F
		1	2	
A	600	14	294	***2.89
	900	14	294	***3.86
	1200	14	294	***4.37
B	600	14	364	***3.32
	900	14	357	***5.10
	1200	14	329	***5.01

If hyperventilation occurs, ventilation coefficient is definitely increased when oxygen consumption is still adequate.

The mean group values of ventilation coefficient are shown in Tables 12. Table 13 shows that there are significant differences between some of the groups. It can generally be concluded that the increase of differences is not the same as in pulse reaction with increasing loads. An increase is observed from 600 to 900 kg-m/min but not usually from 900 to 1200 kg-m/min. Statistically verified discrepancies from group 14 (Tables 14) are observed at 600 kg-m/min with groups 1, 9 and 10. At 900 kg-m/min with groups 1 and 8 (heart cases), 10 (lung cases), and with group 9. At 1200 kg-m/min with groups 1, 9, 10, 12 and 16. Probable discrepancies exist with groups 4, 8 (heart cases) at 600 kg-m/min. At 900 kg-m/min with groups 4 and 6 (heart cases), 12 (lung cases) and 16 (athletes). At 1200 kg-m/min with group 8 (heart cases).

TABLE 14 A. *Differences of ventilation coefficient means between the normal and other groups.*  
Corresponding to Table 12 A.

Group	f	600		900		1200	
		D	t	D	t	D	t
1	37	2.2	*2.29	3.3	**3.24	5.6	***4.59
2	33	0.1	0.05	1.1	1.01	1.7	1.35
3	32	0.8	1.10	0.4	0.60	0.5	0.61
4	43	2.0	*2.15	2.0	*2.45	1.4	1.87
5	44	0.3	0.35	0.8	0.98	0.1	0.99
6	49	1.3	1.71	1.6	*2.13	1.2	1.60
7	49	0.5	0.10	0.7	1.09	0.6	0.74
8	44	1.5	*2.08	2.6	***3.71	3.2	*2.60
9	78	2.2	**3.02	2.2	***3.52	2.1	**2.80
10	47	2.0	*2.36	2.6	**2.90	2.9	**2.94
11	33	-0.2	0.22	-0.1	0.13	-0.1	0.07
12	36	1.2	1.40	2.0	*2.20	2.4	*2.28
13	72	0.2	0.33	0.4	0.39	0.9	1.48
16	35	-1.3	1.94	-1.4	*2.12	-2.4	**2.82

TABLE 14 B. *Differences of ventilation coefficient means between the normal and the other groups.*

*Corresponding to Table 12 B.*

Group	600			900			1200		
	f	D	t	f	D	t	f	D	t
1	44	2.8	**2.75	42	4.2	**3.42	41	5.5	***4.58
2	38	3.0	1.32	37	2.2	1.22	36	1.6	1.28
3	36	0.9	1.32	35	0.5	0.48	35	0.4	0.50
4	51	1.6	1.78	47	1.9	1.72	49	0.9	1.23
5	51	1.0	1.16	50	1.5	1.24	48	0.4	0.41
6	55	1.9	*2.51	53	1.7	1.59	52	1.1	1.51
7	53	0.4	0.61	54	0.6	0.61	54	0.7	0.92
8	52	1.7	*2.12	48	2.7	*2.54	48	3.0	*2.56
9	93	2.3	***3.10	92	2.4	*2.50	85	2.1	***2.98
10	57	3.4	***3.81	54	2.7	*2.39	51	2.8	***2.86
11	38	—0.1	0.12	40	—0.5	0.49	38	—0.4	0.51
12	45	1.0	1.30	40	1.8	1.62	40	2.3	*2.41
13	89	0.1	0.18	83	0.2	0.22	82	0.6	1.07
16	39	—1.3	1.99	46	—1.5	1.61	48	—2.5	***2.98

#### 4. Respiratory rate.

Unfortunately determinations of respiratory rate at work were not taken up until about half the material had been covered, so that respiratory rates are measured only in 243 cases. This has meant that in some groups only few values of respiratory rates are available. Therefore some uncertainty is involved in the calculations of differences between some groups. Nevertheless, the use of the t-test in the statistical treatment of the material compensates this to a large extent.

In Table 15 A and B the group averages are tabulated. From Table 16 A it can be seen that the differences between the groups in general are significant and increase slightly with the load. When as in Table 16 B, all values are taken into account, the differences are highly significant. A comparison between the normal group

TABLE 17 B. *Differences of respiratory rate means between the normal and other groups.*

*Corresponding to Table 15 B.*

Group	600			900			1200		
	f	D	t	f	D	t	f	D	t
3	23	2.4	—	24	2.5	1.97	23	2.2	0.96
5	33	3.0	1.88	33	4.1	1.98	32	3.9	1.73
7	38	2.2	1.80	39	1.8	1.44	37	2.8	1.71
8	34	4.4	**3.47	33	3.9	*2.07	33	4.4	1.70
10	53	6.8	***3.64	51	6.9	***4.65	46	7.1	***4.31
11	36	1.9	1.52	36	0.8	0.93	33	1.9	0.09
12	40	3.0	*2.69	36	3.5	**2.86	34	4.4	**2.97
13	87	4.2	***4.33	87	4.5	***4.73	80	3.3	*2.73

TABLE 18. *The connection between ventilation coefficient (V. c.) and respiratory rate.*

*Cases with values at each of the three loads.*

V. c.	Respiratory rate								
	600			900			1200		
	n	M	s	n	M	s	n	M	s
12	2	14.5	0.7						
15	36	17.2	2.5	24	19.0	3.0	14	20.6	3.4
18	72	19.9	3.5	74	22.1	3.7	47	24.7	4.2
21	41	23.0	3.9	42	26.0	4.0	70	27.5	4.4
24	6	25.3	6.4	12	29.0	4.9	20	31.4	3.8
27	2	32.5	12.1	7	29.0	7.4	8	33.4	5.6
30							6	37.3	5.4
33							4	36.3	1.5
$r=0.55 \pm 0.05$			$r=0.61 \pm 0.05$			$r=0.63 \pm 0.04$			

TABLE 16. *Analysis of variance of respiratory rates between and within groups.*

*A. Corresponding to Table 15 A.*

*B. Corresponding to Table 15 B.*

	Load	Degrees of freedom		F
		1	2	
A	600	14	186	**2.53
	900	14	186	**2.63
	1200	14	186	***2.97
B	600	14	215	***4.32
	900	14	210	***3.34
	1200	14	190	***3.04

TABLE 17 A. *Differences of respiratory rate means between the normal and other groups.*

*Corresponding to Table 15 A.*

Group	f	600		900		1200	
		D	t	D	t	D	t
1	26	3.7	*2.73	3.5	*2.92	10.2	***8.07
2	26	-0.1	0.08	0.8	0.75	2.4	1.19
3	23	2.4		1.5		0.2	
4	30	2.7	1.02	2.4	0.99	1.9	0.70
5	32	2.6	1.62	3.1	1.53	3.9	1.73
6	25	4.1	1.85	4.5	0.94	6.2	1.20
7	36	1.7	1.45	1.4	1.04	2.7	1.63
8	33	3.1	*2.30	3.9	*2.07	4.4	1.70
9	32	3.7	*2.45	4.2	*2.21	3.1	1.27
10	46	5.4	***3.43	6.1	***4.22	7.1	***4.31
11	33	2.2	1.43	0.8	0.83	1.9	0.09
12	33	2.6	*2.14	2.6	*2.37	3.4	**2.75
13	79	1.6	1.61	2.2	*2.27	2.8	*2.31
16	31	-1.2	0.96	-1.9	1.60	-2.4	1.58

TABLE 17 B. Differences of respiratory rate means between the normal and other groups.

Corresponding to Table 15 B.

Group	600			900			1200		
	f	D	t	f	D	t	f	D	t
3	23	2.4	—	24	2.5	1.97	23	2.2	0.96
5	33	3.0	1.88	33	4.1	1.98	32	3.9	1.73
7	38	2.2	1.80	39	1.8	1.44	37	2.8	1.71
8	34	4.4	*3.47	33	3.9	*2.07	33	4.4	1.70
10	53	6.8	**3.64	51	6.9	**4.65	46	7.1	**4.31
11	36	1.9	1.52	36	0.8	0.93	33	1.9	0.09
12	40	3.0	*2.69	36	3.5	*2.86	34	4.4	*2.97
13	87	4.2	***4.33	87	4.5	***4.73	80	3.3	***2.73

TABLE 18. The connection between ventilation coefficient (V. c.) and respiratory rate.

Cases with values at each of the three loads.

V. c.	Respiratory rate								
	600			900			1200		
	n	M	s	n	M	s	n	M	s
12	2	14.5	0.7						
15	36	17.2	2.5	24	19.0	3.0	14	20.6	3.4
18	72	19.9	3.5	74	22.1	3.7	47	24.7	4.2
21	41	23.0	3.9	42	26.0	4.0	70	27.5	4.4
24	6	25.3	6.4	12	29.0	4.9	20	31.4	3.8
27	2	32.5	12.1	7	29.0	7.4	8	33.4	5.6
30							6	37.3	5.4
33							4	36.3	1.5
	$r=0.55 \pm 0.05$			$r=0.61 \pm 0.05$			$r=0.63 \pm 0.04$		

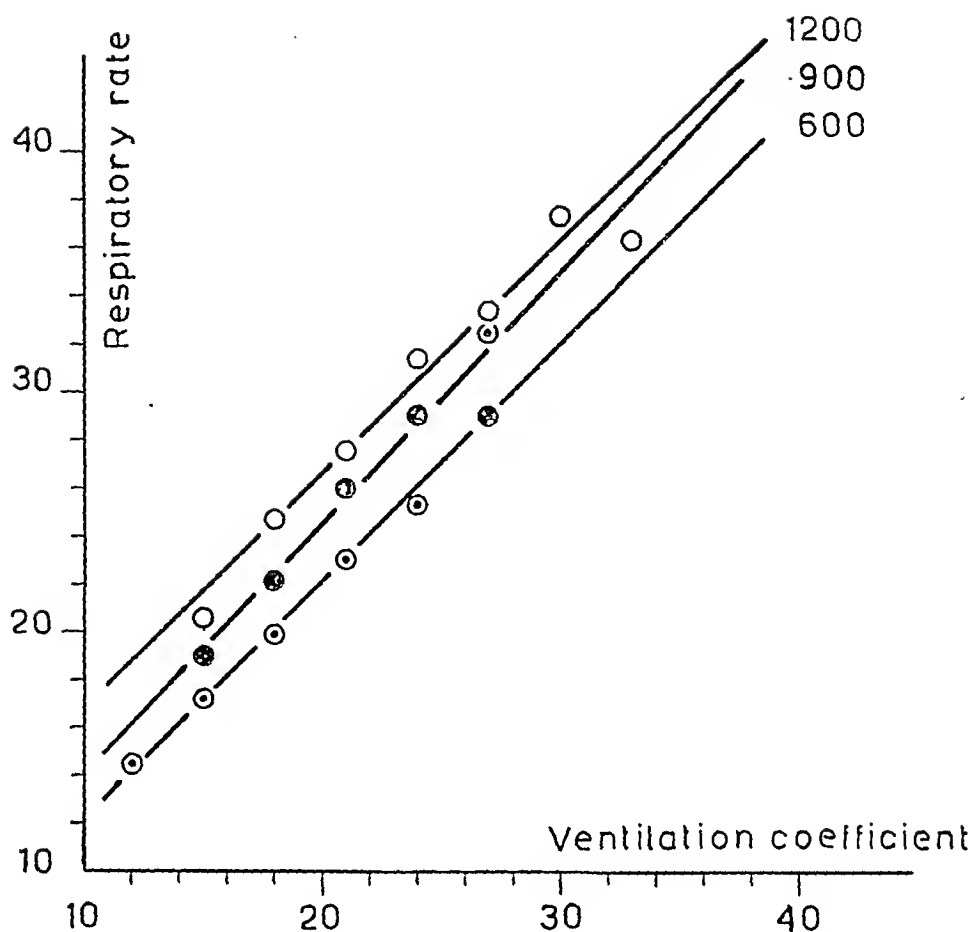


Fig. 2. *The connection between ventilation coefficient and respiratory rate. Mean values of respiratory rate for classes of ventilation coefficient with regression lines.*

(14) and the other groups yields significant discrepancies, at least at the higher loads with groups 1 (heart cases) and 10, 12, 13 (lung cases). (Table 17 A and B).

Although the corresponding differences for ventilation coefficients are also significant in some other groups, there are similarities when compared to the respiratory rate differences. An analysis of the connection between ventilation coefficient and respiratory rate gives moderate linear correlations at the three loads (Table 18 and Fig. 2). The correlation increases slightly with the load. Correlation between total ventilation and respiratory rate is of the same magnitude.



## 5. Significance of vital capacity.

Average values of vital capacities for the various groups are shown in Table 19. Analysis of variance of the differences between the group means gives an F-value of 7.32\*\*\*. The lowest averages in the groups are apparent in that of valvular disease, and of emphysema. The highest values are in the hypernormal groups 15 and 16.

TABLE 19. *Vital capacity (liters).*

Group	n	M $\pm$ m	Group	n	M $\pm$ m
1	16	3.6 $\pm$ 0.16	9	68	4.3 $\pm$ 0.08
2	11	4.3 $\pm$ 0.24	10	37	3.7 $\pm$ 0.17
3	8	4.3 $\pm$ 0.20	11	16	4.3 $\pm$ 0.17
4	22	4.2 $\pm$ 0.16	12	20	3.9 $\pm$ 0.14
5	25	4.3 $\pm$ 0.14	13	67	4.6 $\pm$ 0.08
6	27	4.3 $\pm$ 0.10	14	37	4.4 $\pm$ 0.13
7	29	4.6 $\pm$ 0.19	15	26	4.7 $\pm$ 0.11
8	29	4.4 $\pm$ 0.15	16	19	5.0 $\pm$ 0.13
F=***7.32					

As ventilation and respiratory rate may be influenced by vital capacity, it is of interest to investigate the correlations between these variables. The correlation coefficients between vital capacity and respiratory rate are rather small (Table 20 and Fig. 3), but there is a clear tendency to higher respiratory rates with decreasing vital capacities. It may be observed that the range of variation in most vital capacity-groups is fairly large. The correlation between vital capacity and ventilation coefficient is slightly less than for respiratory rate.

In addition to other factors, total ventilation is limited by vital capacity and by respiratory rate. When vital capacity is low, total ventilation is more easily maximized with increasing respiratory rate. When vital capacity is normal, or above normal, total ventilation may be maximized when the respiratory rate

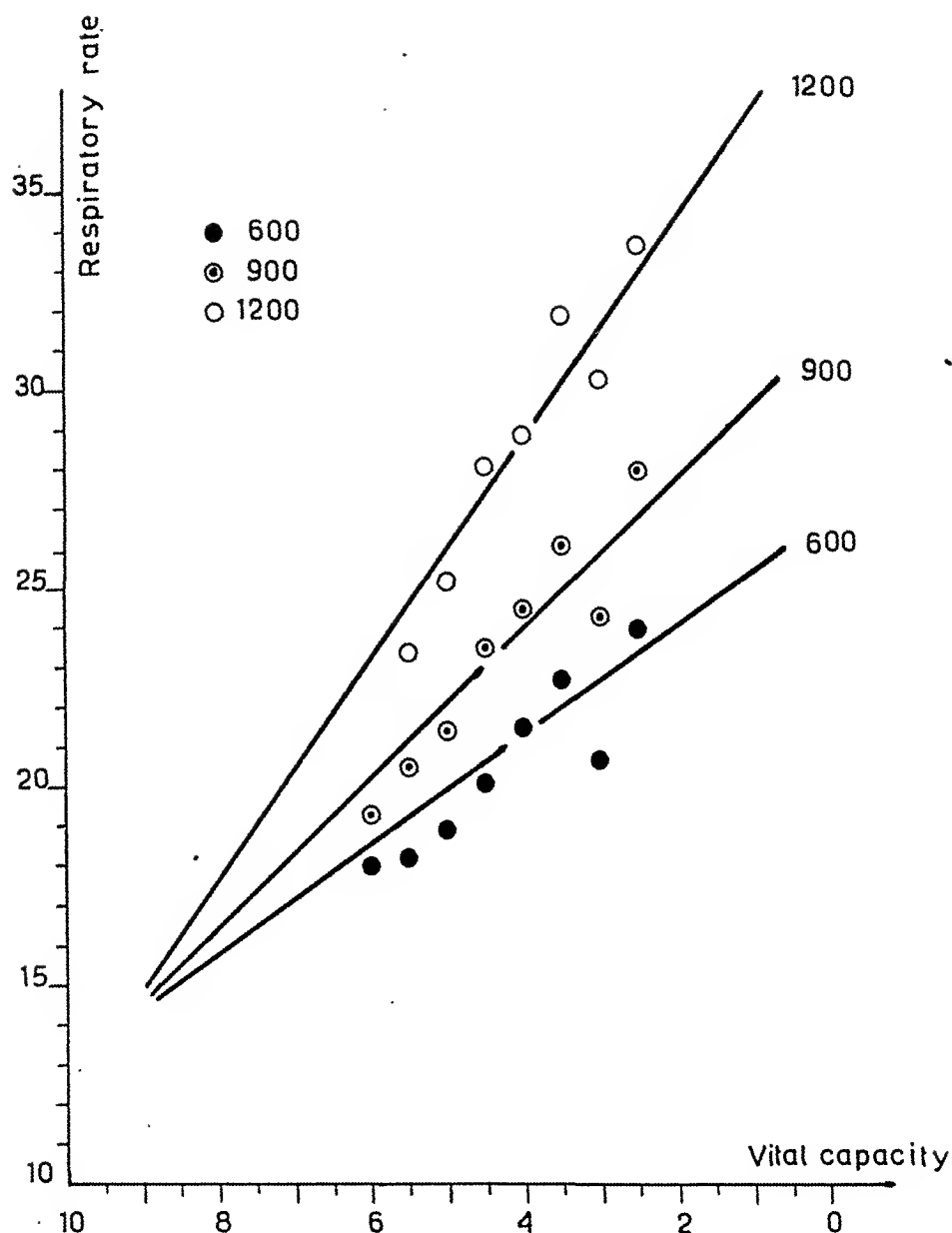


Fig. 3. The connection between vital capacity and respiratory rate. Mean values of respiratory rate for classes of vital capacity with regression lines.

reaches greater values. This explains why no simple correlation is found between ventilation coefficient and vital capacity.

*The results of the group-analysis above can be summarized as follows.*

TABLE 20. *The connection between vital capacity and respiratory rate.*

*Cases with values at each of the three loads.*

V. cap.	n	Respiratory rate					
		600		900		1200	
		M	s	M	s	M	s
2.5	3	24.0	1.7	28.0	2.7	33.7	0.6
3.0	11	20.7	2.9	24.3	2.7	30.3	3.4
3.5	18	22.7	6.0	26.1	6.6	31.9	6.7
4.0	38	21.5	4.3	24.5	4.7	28.9	4.8
4.5	53	20.1	4.2	23.5	4.6	28.1	5.4
5.0	42	18.9	3.9	21.4	4.1	25.3	5.0
5.5	21	18.2	3.5	20.5	4.6	23.4	5.1
6.0	8	18.0	4.1	19.3	5.6	21.0	4.7
6.5	1	16		21		24	
7.0	1	20		20		21	
		$r = -0.30 \pm$		$r = -0.32 \pm$		$r = -0.40 \pm$	
		$\pm 0.06$		$\pm 0.06$		$\pm 0.06$	
		F = **2.79		F = ***3.78		F = ***6.70	

Oxygen consumption. *No significant differences between the group means at any load.*

Pulse rate. *Significant differences increasing with the load. Taking the group of ordinary healthy subjects as a basis, the differences are shown to exist in some of the heart groups and hypernormal groups.*

Ventilation coefficient. *The tendency to increasing differences with load is not conspicuous to the same extent. Significant differences are shown to exist in some heart groups, and in emphysema groups.*

Respiratory rate. *There is no striking tendency of increasing differences with load. Significant differences are observed in the group of rheumatic valvular disease, and in groups of pulmonary diseases.*

## VI. Determination of the individual working capacity.

### 1. General discussion.

Although the response to work of the various functions investigated show clear differences between normal subjects and some heart and lung patients, as well as between the latter, nothing can be said about the individual working capacity from such group-calculations.

The regularity of the variations with work-load of pulse and respiratory rates seem to be the functions best suited for this purpose. In addition, pulse and respiratory rates exert an influence upon cardiac output and total ventilation respectively. *Pulse rate* is roughly a linear function of oxygen consumption and work-load. Judged by the findings referred to in the survey of literature it is usually 170—200 at exhausting work in *adult* healthy subjects. At these values no further increase of cardiac output is to be expected with increased pulse rate. However, oxygen for the working muscles may still be supplied by increased utilization, but it is only possible to work for a short time under such conditions. The factor responsible for limiting cardiac output is the time of diastole which at pulse rates above 180 may be too short for adequate filling of the heart.

It is thus possible to make an estimate of the limit to which cardiac output can be increased, by studying the pulse curve during work in any individual case. When the pulse rate has reached a sufficiently high value at a certain load, this load is said to be maximal. In this study the maximum heart rate at which work can be performed adequately is put at 170 beats per minute. If the pulse rate at any load is above this value, the load is regarded as an overload. It must be admitted that some individuals can work with a pulse rate slightly above 170 beats per minute, but they are not expected to do this for any length of time as there is usually a prolonged increase of pulse rate. Consequently they may reach a value of 180 or more after some time and are thus at the limit of their capacity.

If an individual has not reached 170 beats per minute at the loads employed, his maximum work-load can be estimated from the relation between work-load and pulse rate. If the pulse rate at the 6th minute of work is just below 170, it may be difficult to say whether the load is to be considered maximal or not. The following procedure has been used in such cases. When the pulse rate level is fairly constant, the subject is said to work adequately, but when the pulse rates at the load in question are increasing so that the next value is expected to be above 170, the load is regarded as maximal.

*Respiratory rate* likewise shows a regular increase with load. It appears however to be a less stable factor than pulse rate. At exhausting work, values above 30 resp. per minute have usually been found by previous investigators. The range of variation is wide. A value above 30 is however regarded as a sign that the subject has reached the limit of his capacity with respect to his respiratory ability. Respiratory rate exerts a limiting influence upon total ventilation, for when it is sufficiently high, no increase of ventilation is to be expected. It is difficult to say at which value this effect occurs in the individual case, as vital capacity, dead space and residual air are important factors in the mechanism. For practical purposes, a respiratory rate of 30 can be used as the highest value, definitely consistent with a good performance. Values above 30 show that the load is, or begins to be, too great. This limit is concluded from previous investigations, and from estimation of the degree of distress produced by work on the persons in the present material. This method of evaluating the respiratory response is with any greater degree of certainty, only valid when the respiratory rates at the different loads behave in the usual manner. There are cases that have values of about 30 at light loads, but no, or only slight increase occurs with load, sometimes even a decrease being observed. Such cases are difficult to appraise, but often psychic influences can be suspected. No attempt has been made to estimate working capacity from extrapolation of respiratory rates, as the differences between the values of the various loads are usually numerically small. An extrapolation could therefore be considerably erroneous.

The ventilation coefficient at the beginning of this study was believed to give valuable information as to the respiratory response to work (Wahlund, 1945). A definite increase of ventilation coefficient was to indicate the increase of total ventilation which accompanies exhaustion. As discussed before, it is not necessary that such an increase occurs if for instance respiratory rate is sufficiently high. This effect is more pronounced when conditions are such that alveolar ventilation is decreased. Another statement also made at the beginning of this study, that a ventilation coefficient above 24 indicates a lesser capacity to work, will be justified to some extent in later pages.

Before discussing the individual working capacities of the persons investigated, according to pulse rate and respiratory rate analyses, it is of interest to show that there is on the whole some connection between the pulse rate and respiratory rate levels. When for 1200 kg-m/min respiratory rates are correlated to pulse rates in any individual case, it is found that they increase with each other, although the range of variation is fairly wide (Table 21, Fig. 4), whereas no definite correlation is found between ventilation coefficient and pulse rate.

TABLE 21. *The connection between pulse rate and respiratory rate at 1200 kg-m/min.*

Pulse rate	Respiratory rate		
	M	s	n
120	23.5	3.6	5
130	24.8	7.2	10
140	25.8	5.0	20
150	25.8	6.4	49
160	27.2	5.4	49
170	28.8	4.5	48
180	29.3	6.3	16
190	32.6	4.4	7

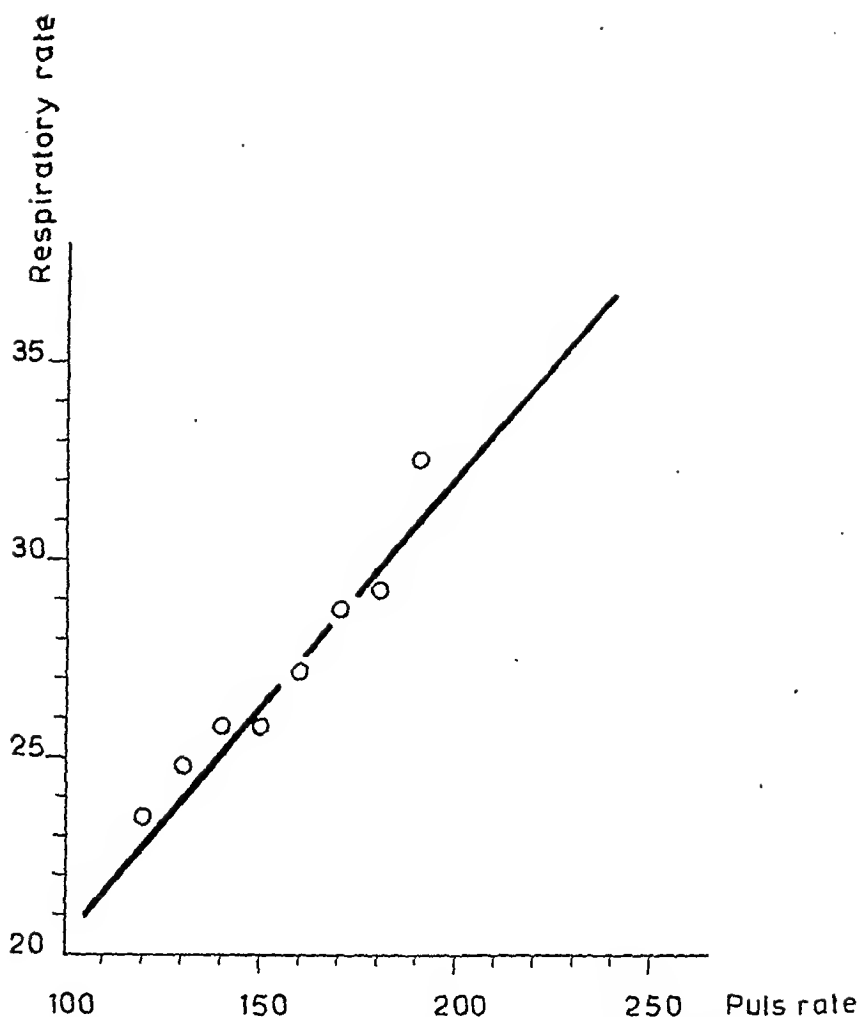


Fig. 4. The connection between pulse rate and respiratory rate at 1200 kg-m/min. Mean values of respiratory rate for classes of pulse rate with regression line.

To summarize, working capacity is determined in the following manner in most cases.

1. Circulatory reaction. The highest load at which pulse rate does not exceed 170 beats per minute.
2. Respiratory reaction. The highest load at which respiratory rate does not exceed 30 respirations per minute.

## 2. General results.

In Tables 22 maximal loads are presented for all individuals in the various groups. Let us first consider the group of ordinary healthy subjects (14). The pulse rate analysis (Table 22 A) shows that 87 % of the subjects had a working capacity of at least 1200 kg-m/min. The remainder had a capacity of 900 kg-m/min. The respiratory rate analysis generally yields the same result. When the capacity is determined with respect to both respiratory and circulatory responses (Table 22 B and C), a slightly lower percentage is observed for 1200 kg-m/min. Fig. 5 shows the reactions of a normal subject. In the group of hypernormal subjects (16) no one had a capacity of less than 1200 kg-m/min, whereas in group 15, 8 % had a capacity of 900 kg-m/min.

TABLE 22 A. *Working capacity.*  
*According to pulse rate analysis.*

Group	n	Working capacity, kg-m/min.							
		300	600	900	1200	1500	1800	≥ 2100	≥ 1200
1	16	1	6	9					
2	11	1	3	3	3	1			4
3	8			4	4				4
4	23		1	7	10	3	2		15
5	25	2	3	8	10	1		1	12
6	27	2	9	9	5		2		7
7	29		1	8	12	8			20
8	29	1	3	9	10	6			16
9	68		10	31	18	8	1		27
10	36	1	3	8	17	3	2	2	24
11	16			3	9	4			13
12	20		1	6	3	7	2	1	13
13	67		2	22	30	12	1		43
14	40			5	30	4		1	35
15	26			2	4	11	6	3	24
16	27				6	9	4	8	27



TABLE 22 B. *Working capacity.*  
*According to respiratory rate analysis.*

Group	n	Working capacity, kg-m/min.				
		< 300	300	600	900	≥ 1200
1	4				4	
2	4					4
3	2					2
4	8					8
5	11			2	2	7
6	3			1	1	1
7	16				3	13
8	11				5	6
9	10			1	3	6
10	37	4	5	3	11	14
11	15			1	3	11
12	19			1	7	11
13	65			3	13	49
14	24				4	20
16	9					9

Of course it is somewhat inadequate to speak of *one normal* working capacity. The ability of healthy men to work is influenced by several factors; for instance daily occupation and the extent to which they practise athletics. A man sitting all day in his office is not necessarily as capable of physical work as a labourer, but they may each have the «normal» working capacity of their respective occupational group.

The majority of cases in this investigation have been exposed to some kind of training, either in their daily occupation or during military service. They may thus be regarded as homogeneous in this respect. Furthermore a load of 900 kg-m/min, corresponding to an overconsumption of 1.5—2 liter of oxygen per minute, cannot be considered as a severe load for ordinary subjects. 1200 kg-m/min could not be performed by some «normal» subjects but as the majority of the persons in the groups of healthy subjects could sustain this load, it is here referred to as

TABLE 22 C. *Working capacity.*  
*According to pulse and respiratory rate analysis.*

Group	n	Working capacity, kg m/min.				
		< 300	300	600	900	≥ 1200
1	4			1	3	.
2	4				2	2
3	2					2
4	8				1	7
5	11			2	2	7
6	3			1	1	1
7	16				3	13
8	11				7	4
9	10			1	5	4
10	36	3	5	3	13	12
11	15			1	3	11
12	19			2	8	9
13	65			4	23	38
14	24				5	19
16	9					9

the normal working capacity. A capacity of 900 kg-m/min is said to be slightly less than normal and capacities below 900 kg-m/min definitely abnormal.

Table 22 A shows that the working capacities differ considerably within the groups when pulse curve analysis is the basis of estimation. High percentages of low capacities are seen in the groups which are definitely separated from the normal group with respect to pulse rate levels. There are however also cases in the other groups that have abnormal capacities, although the pulse level group-analysis at the different loads did not give abnormal results. The same can be seen in table 22 B regarding respiratory rate analysis.

Group-analyses are necessary for solving some problems, but when, as in this case, some groups consist of cases with high pulse levels, and a sufficiently large number with low pulse levels, no differences need occur between the pulse means. Nevertheless

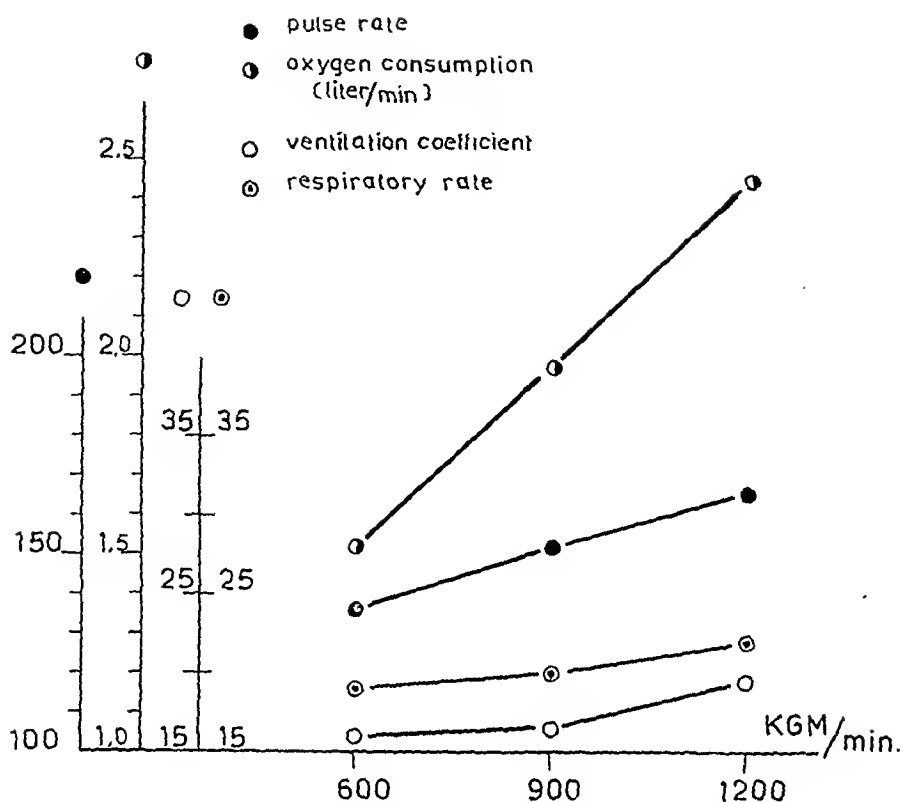


Fig. 5. Pulse and respiratory rates, ventilation coefficient and oxygen consumption at the various loads in a normal subject.

definite abnormal reactions exist in the groups. Compare for instance the pulse rate differences in Table 7, with the working capacities in Table 22 A in groups 4, 5, 7, 8 and 10.

The results in Tables 7 and 17 show that marked differences are found in groups containing cases which in advance could be regarded as less capable of work when compared to ordinary subjects. This is in accordance with the results of other investigators on the reactions of different functions to work. In most tests it is however not possible for a more able subject to distinguish himself above other subjects than those that are definitely abnormal. When for instance pulse or respiratory rates are used at a single light load as indicators of working capacity, nothing is known about the reaction at higher loads. The only possibility

to draw conclusions from one light load should be based on the assumption that all individuals react in the same manner as regards the increase of pulse or respiratory rate with load. Table 8 shows however that this is far from true when pulse rates are considered.

In Tables 22 it is seen that with increasing load a more differentiated picture of the working capacities of the individuals is attained. If the test had been restricted to 300 or 600 kg-m/min, only a few of the cases could, with the criteria used, be distinguished from the others. At a load of 900 kg-m/min a larger number of cases are selected and at 1200 kg-m/min still more are weeded out.

*By studying the pulse and respiratory rate response of an individual to a series of loads, it is thus possible to get more detailed information of the individual working capacity than by using only one load.*

TABLE 23. *The connection between working capacities estimated according to pulse and respiratory rate.*

Pulse analysis	Resp. analysis				
	< 300	300	600	900	≥ 1200
300		1			
600	1	1	4	2	
900		1	2	31	24
≥ 1200	2	2	6	21	125

Most of the capacities above 1200 kg-m/min in Table 22 A are found by extrapolation from the pulse curves. As mentioned before no extrapolation has been tried with respiratory rates. The distribution of the capacities in Tables 22 B and C are seen to be about the same, which indicates that, up to a capacity of 1200 kg-m/min, the pulse rate analysis gives about the same result as that of respiratory rate. In fact, the rank correlation coefficient calculated on the basis of the two methods of estimating capacity is approximately unity. A suspicion of this could be drawn from

Table 21, in which the respiratory rates were seen to increase with the pulse rates. The range of variation of respiratory rates is however rather wide in each pulse group. A thorough examination of the correlation between the capacities determined with either method, reveals that those cases which have greater differences between the methods belong to the pulmonary groups. They have by respiratory rate analysis a lower capacity than by pulse rate analysis. Table 23 shows the distribution of capacities.

TABLE 24. *The connection between working capacity and ventilation coefficient. A according to pulse rate, B to respiratory rate analysis. C according to pulse and respiratory rate analysis.*

Working capacity	Number of cases with vent. coeff.		
	< 22.5	22.5—24.4	≥ 24.5
A. 300		1	1
600	10	8	11
900	71	12	21
≥ 1200	191	12	16
B. 300	1		1
600	2	2	3
900	17	5	17
≥ 1200	123	7	5
C. 300	1		1
600	3	3	3
900	37	4	18
≥ 1200	102	7	4

In a study on normal subjects Szakall (1944) found that the ventilation coefficient at heavy work was above 24 when the subjects had a poor working capacity.

When ventilation coefficients are correlated to working capacities no definite relation appears in this material. Table 24 shows that high coefficients seem to indicate low capacities, whereas low coefficients are connected with both high and low capacities. High values of the ventilation coefficient may thus indicate poor working capacity, but »normal» values cannot be used as indicators.

### 3. Survey of results regarding working capacities in the various groups.

In group 1 no one had a working capacity above 900 kg-m/min. 13 cases had mitral valvular disease, the remainder aortic diseases and all but one were compensated at rest. Case 142, with the least working capacity (300 kg-m/min), was apparently just on the limit of decompensation. The three aortic cases had capacities of 900 kg-m/min. No correlation between heart volumes per square meter body surface and working capacity is found. The number of cases in this group are however too few for definite conclusions of such correlation. The heart volume was not determined in one case, and in the others all but one showed volumes above 450 ml per square meter body surface. In Fig. 6 the reactions of a typical heart case are shown (Case 189).

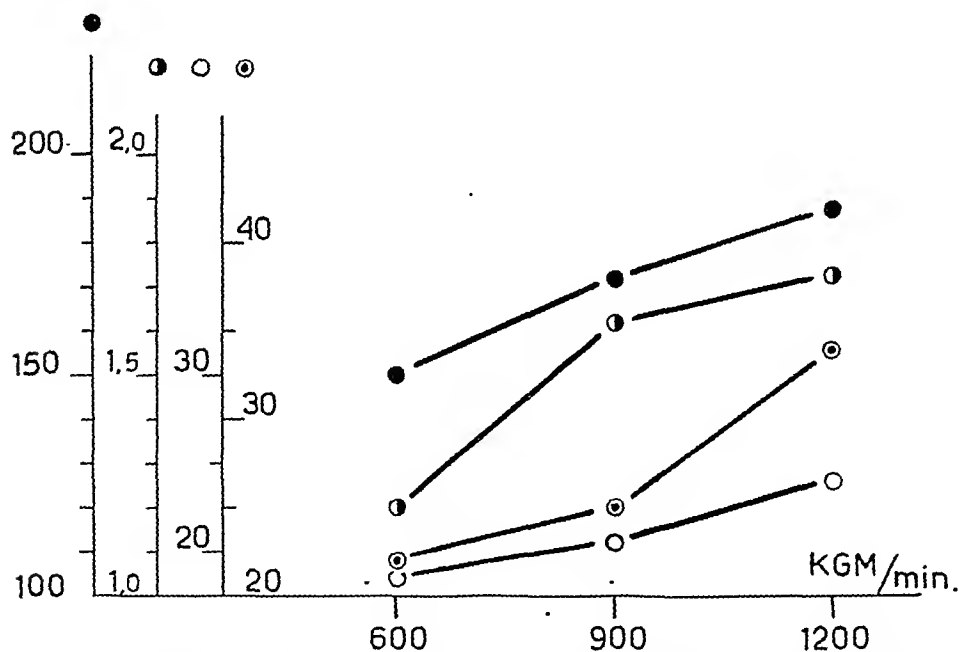


Fig. 6. Pulse and respiratory rates, ventilation coefficient and oxygen consumption at the various loads in a case of rheumatic valvular disease.

Signs as in Fig. 5, p. 58.

In group 2 there are 2 cases (101 and 187) with coarctation of the aorta (both having a working capacity of 900 kg-m/min), 5 cases with septal defects (one case 300 kg-m/min, one 600, one

900 and two cases 1200 kg-m/min), and 4 cases with patent ductus arteriosus (two cases 600 kg-m/min and two 1200 kg-m/min or more). There is no definite correlation between working capacity and heart volume among these few cases. The largest hearts (490—560 ml pr square meter body surface) were found in 3 cases with septal defects. The cases with patent ductus arteriosus showed normal heart volumes.

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In group 3 there are 5 cases suspected of septal defects (3 having working capacities of 900 and 2 of 1200 kg-m/min) and 3 cases suspected of patent ductus arteriosus (one having 900 kg-m/min and two 1200 kg-m/min). The cases in group 3 showed normal heart volumes.

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In group 4 all but 9 cases had heart volumes below 450 ml per square meter body surface. The configurations of the enlarged hearts were however not definitely consistent with valvular disease. The largest heart volumes were not connected with abnormal working capacity.

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Of the cases with moderate hypertension in group 5, one half have normal capacities, but no correlation is found to blood pressure, x-ray or electrocardiographic findings.

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In group 6 cases with acute myocarditis as well as suspected cases are included. Some cases are only slightly suspected. 4 cases in this group have no definite suspicion of myocarditis but had infection of the upper respiratory system. These 4 cases had abnormal working capacities. In general working capacity is correlated to the severity of the symptoms. No patient with acute myocarditis was tested in the most acute stage of disease.

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No reasons for enlargement of the heart have been discovered for the patients in group 7. In some cases hypertension could be suspected, but the blood pressure values were not abnormal at the time of examination. 24 cases had heart volumes of 460—500 ml

per square meter body surface. In 8 of these cases (cases 4, 98, 103, 150, 227, 285, 290, 367) the increased heart volume was caused by enlargement of the left ventricle; 3 of these 8 cases (4, 98, 150) had a working capacity of 900 kg-m/min, and the rest had 1200 or more. Of the remaining 16 cases in this heart volume group, 5 had a working capacity of 900 kg-m/min, and 11 of 1200 or more. One case with a heart volume of 550 had a working capacity of 1200 kg-m/min.

In 4 cases with heart volumes of 560—600 ml per square meter body surface, 3 had a working capacity of 1200 kg-m/min or more (2 were suspected of hypertension, 277, 329), whereas one had 600 kg-m/min. This last case (113) showed a slight suspicion of having had a myocarditis.

*It is concluded that a heart volume above normal need not necessarily be accompanied by a definitely abnormal working capacity, at least not at some periods of the «disease».*

Group 8 contains cases with electrocardiographic abnormalities.

- a) Disturbance of atrioventricular conduction (7 cases).
- b) Disturbance of intraventricular conduction (7 cases).
- c) Pre-excitation (WPW electrocardiogram) (2 cases).
- d) Signs of coronary insufficiency (9 cases).
- e) Signs of orthostatic reaction (signs of «coronary insufficiency» in standing position) (4 cases).

a). 2 cases had marked prolongation of the PQ time (0.24 sec), one of which (214) showed abnormal working capacity, PQ time after work was prolonged but varying. One of the cases was tested after administration of atropine subcutaneously, and had practically normal values. 3 cases had moderately prolonged PQ time (0.21—0.24 sec), 2 of which showed normal values after work, whereas one was unchanged. All had capacities of at least 1200 kg-m/min.

The remaining 2 cases had no PQ prolongation at rest, but after work the PQ time was somewhat prolonged; in one case with a working capacity of 900 kg-m/min from 0.18 to 0.20 sec, in the other with a capacity of above 1200 kg-m/min from 0.20 to 0.21 sec. This last difference is not however quite reliable as it falls



within the error-limits of the apparatus. But as a rule the PQ time in healthy subjects is usually some hundredths of a second shorter after moderate work than at rest.

In addition, 4 athletes in group 16 had abnormal PQ values. As they were otherwise normal at the examination and had no heart trouble they were not excluded from group 16, and will therefore be discussed with that group.

b). 6 cases with slight signs of intra-ventricular conduction disturbance (QRS  $> 0.10$  sec., notching of QRS) all had normal working capacity except one (600 kg-m/min). One case with intraventricular block of the Wilson type had normal working capacity.

c). One case had a working capacity of 600 kg-m/min, the other was normal.

d). These cases had one or more of the following electrocardiographic abnormalities and usually subjective symptoms of coronary insufficiency, low or inverted T-waves or depressed ST-intervals. One case (45) was discovered at a hypoxemia test with 9 % oxygen, and one had signs only after work.

An abnormal working capacity was found in 6 of the 9 cases in this group (4 having 900 kg-m/min, one 600 and one 300 kg-m/min). This last capacity (300 kg-m/min) was not founded on pulse or respiratory analysis; the man had to stop because of the sudden developement of heart pains after a short period of work at 600 kg-m/min. This constitutes a functional test for patients with anginal symptoms. Their working capacity is limited by other factors than cardiac output or ventilation. Nevertheless the point of non-ability can be determined and indicates the working capacity.

e): 2 of the cases with orthostatic reaction showed working capacities of 900 kg-m/min. The others were normal.

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Group 9 contains cases that had no symptoms or diagnoses that could place them in another group. 2 cases had signs of slight hyperthyroidism; they both had capacities of 900 kg-m/min. 16 cases were of the leptosome body type, all having abnormal working capacity; 10 cases 900 kg-m/min and 6 cases 600 kg-m/min.

Only 2 of them showed signs of muscular fatigue at the highest load performed. In one of the cases slight signs of orthostatic pulse reaction appeared, the same was found in another case in group 9 with normal body type. These last cases both had working capacities of 900 kg-m/min.

In the remaining 22 cases of this group with abnormal working capacity, nothing was found that could explain the apparently reduced ability to work. If the possibility that they have some undiscovered heart or lung disease is excluded, the only explanation is that they are constitutionally less capable. In the group of ordinary healthy subjects (14), a few had working capacities less than 1200 kg-m/min. Group 9 cannot however be regarded as a group of »normal» subjects, as the difference between the groups with respect to the percentage of cases having capacities less than 1200 kg-m/min is  $47.8 \pm 7.9$  % when calculated from pulse curve analysis. The difference based on pulse as well as respiratory rate analysis is  $39.2 \pm 17.5$  %.

It should be mentioned here that the correlation coefficient between body weight and working capacity determined for the total material is  $0.28 \pm 0.04$  and thus definite, but the influence of pathological factors plays a role. The correlation coefficients in groups 9, 14, 15 and 16 are however of the same magnitude (Table 25, Fig. 7).

TABLE 25. *The connection between body weight and working capacity estimated according to pulse rates.*

Body weight	Working capacity			Body weight	Working capacity		
	M	s	n		M	s	n
50	1000	120	7	85	1320	130	23
55	890	90	23	90	1310	130	11
60	1060	130	64	95	1280	171	4
65	1090	110	101	100	1440	150	6
70	1150	110	93	105	1400	210	3
75	1180	120	83	110	1500		1
80	1300	110	41				
$r=0.28 \pm 0.04$				$F=***3.59$			

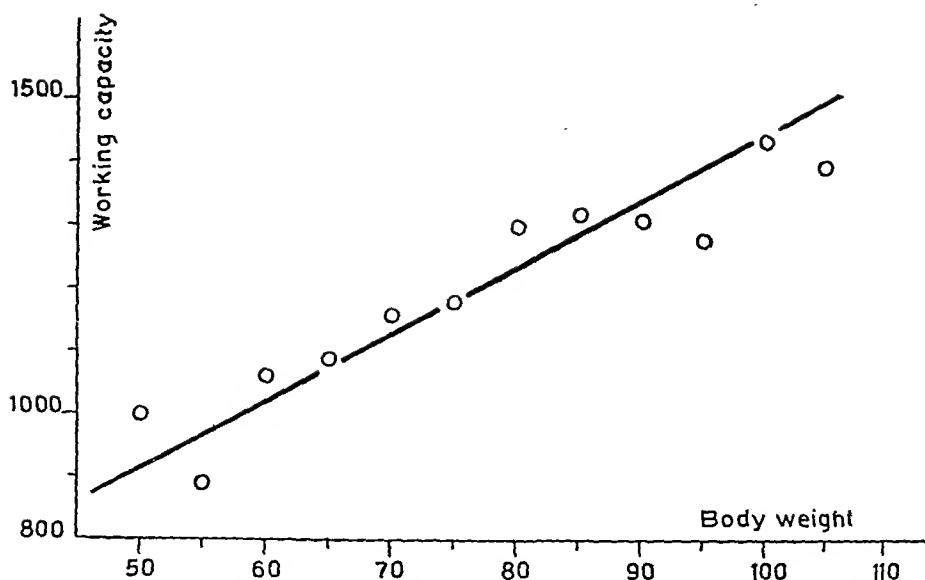


Fig. 7. *The connection between body weight and working capacity. Mean values of working capacity for classes of body weight with regression line.*

The pulmonary cases included in group 10 have signs of heart disease, although the subjective symptoms were not very impressive. The majority were suffering from hypertension of moderate degree connected with electrocardiographic symptoms of coronary insufficiency and enlarged heart volume. Some cases showed enlarged hearts suspected of hypertension, but no increased blood pressure at the time of the examination. A few cases had only moderately enlarged hearts, one a bundle branch block, and one prolonged PQ time at rest but normal after work. The working capacities show a fairly high percentage of values below 1200 kg-m/min, with pulse as well as respiratory analysis.

In groups 11 and 12 the working capacities are distributed in about the same way, with a lower percentage of abnormal cases in group 11.

Typical reactions of a case with emphysema are shown in Fig. 8. (Case 364).

*There is no clear cut correlation of working capacity with the ratio of residual air to total capacity of the lungs or with vital capacity. But the highest residual air ratios (above 45 %) and the*

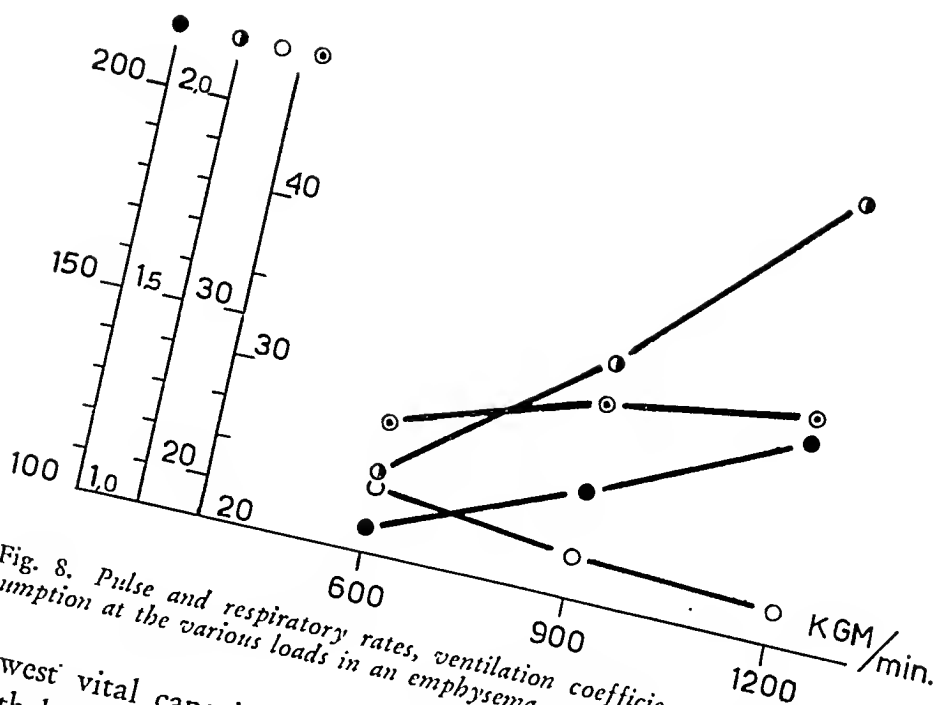


Fig. 8. Pulse and respiratory rates, ventilation coefficient and oxygen consumption at the various loads in an emphysema case. Signs as in Fig. 5, p. 58.

lowest vital capacities (below 3 liters) are definitely connected with low working capacity. However, low capacities occur also with slightly and moderately increased residual air ratios, and with vital capacities above 3 liters.

These results, partly in accordance with the statements made by Hurtado, McCann and Fray (1933) regarding the importance of residual air ratio, show the necessity of making working tests for the proper understanding of the influence played by the degree of emphysema upon the working ability of an individual. It is not at once clear that a subject with a moderate degree of emphysema or a slightly decreased vital capacity has a definitely abnormal working capacity. Likewise it is not clear that a subject with practically normal residual air ratio or vital capacity has a «normal» working capacity. Among such factors exerting an influence upon the ability to perform work. Among such factors age and the presence of chronic bronchitis can be mentioned. This is to some extent demonstrated in group 13, where cases with signs of tracheo-bronchitis, verified by bronchoscopic examination, are included. The patients in this group had normal residual air ratios, and showed working capacity percentages bet-

ween those of groups 11 and 12. The vital capacity values in group 13 were, with a few exceptions, apparently normal (the lowest value was 3.2 liters). The number of lung cases in this investigation is too small for definite conclusions of the influence played by various pathological findings of the lungs upon working capacity.

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In the group of athletes (16) some cases with objective signs of heart abnormalities are included. They had not complained of any troubles at training or work and were discovered at routine examinations at the Health Control Station for Athletes. 4 cases showed prolonged PQ time at rest (141, 218, 450, 469). Case 141 0.30—32 sec; after the test 0.26—27 sec. Case 218 0.21—25 on various occasions; after work 0.21—22. Case 450 at the routine examination 0.30—35 sec, at the time of the working test 0.18—20; after work 0.16 sec. Case 469 at one routine examination showed atrioventricular block II with PQ 0.20—24 (pulse rate 40), 5 months earlier he had a PQ time of 0.17 (pulse rate 50—55). At the working test some days after the AV block II had been discovered he showed normal PQ time (0.16, pulse rate 55—60; after work 0.16).

There was no reason to believe these men to have actual heart disease, and subsequent controls did not reveal any signs of disease.

One case (451) had on one occasion nodal rhythm (sino-auricular block?). Case 1 had pre-excitation electrocardiogram with normalization after work. Case 202 showed a difasic T-wave in standard lead I, the importance of this is difficult to estimate. The man was a cycle champion and still is.

Case 209 was a man with no subjective symptoms but with definite hypertension and slight enlargement of the heart. He has however at the time of the test been regarded as more athlete than hypertensive and is thus included in group 16.

In group 16 there are 4 cyclists and 4 swimmers. The majority of the remainder consist of middle and long distance runners and ski-runners. Apparently no marked differences exist in the reaction at work between subjects performing different types of

athletics. The best performance observed in this investigation was found in case 459, a 5000 m champion, who worked up to 2400 kg-m/min without any signs of fatigue or insufficiency. The reactions of another very able athlete (465) are shown in Fig. 9.

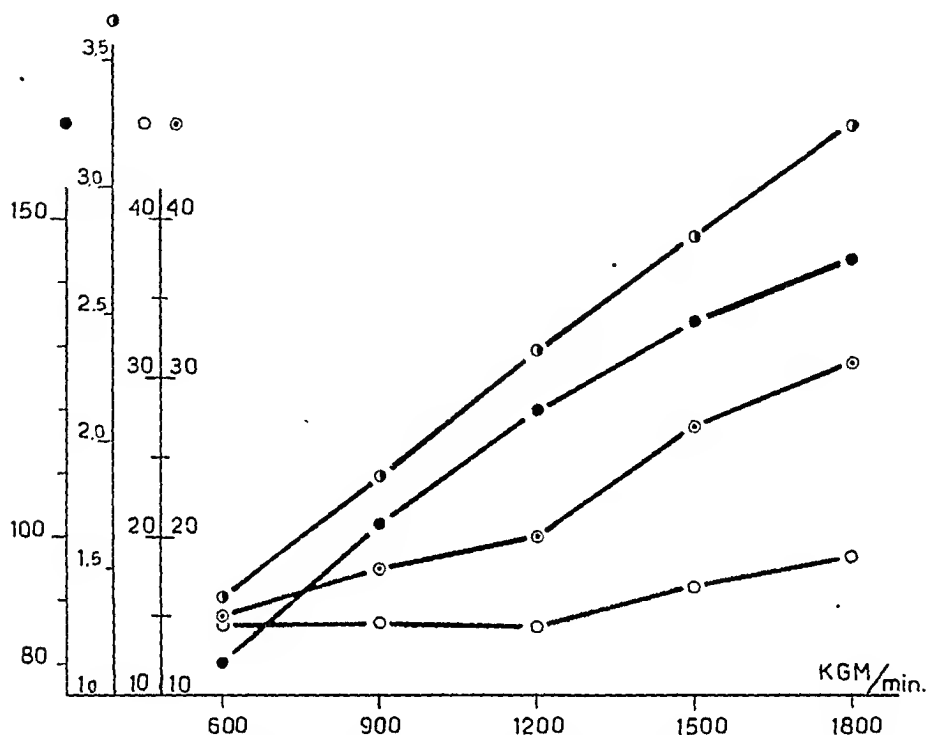


Fig. 9. Pulse and respiratory rates, ventilation coefficient and oxygen consumption at the various loads in an athlete. Signs as in Fig. 5, p. 58.

The subjects in group 16 were in various degrees of training, a few of them should perhaps have been regarded as ordinary healthy subjects at the time of testing, but they had all had some connection with athletics.

The distribution of working capacities in groups 1, 6, 14 and 16 is shown in Fig. 10.

From the present material no definite conclusion can be drawn as regards the influence of age upon working capacity. An examination of that relation should be based on a large number of healthy individuals who are homogeneous with respect to state of training and body type.

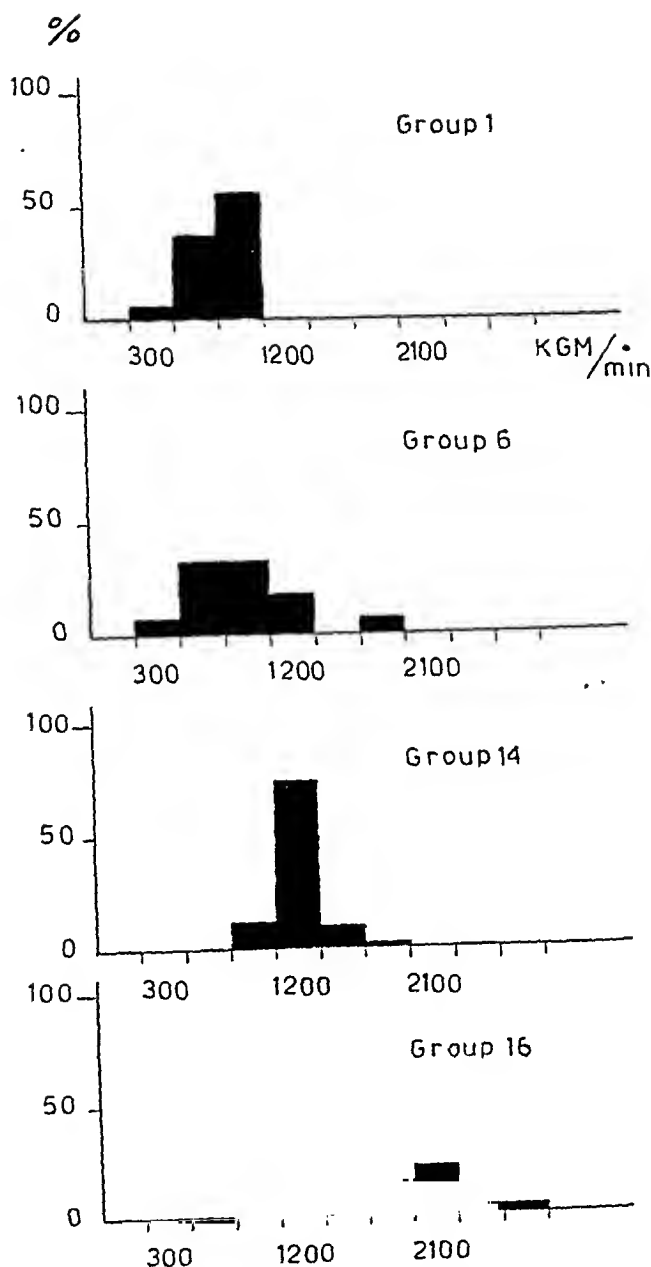


Fig. 10. *Distribution of working capacities in groups 1, 6, 14 and 16. Number of cases expressed as percentages of the respective groups.*

## VII. Conclusions.

When work is performed by normal subjects the reactions of several factors are well defined in any one individual. By studying these reactions at work of varying degree it is possible to get a general view of the type of variation in physiological functions. In non-healthy subjects the reactions are generally speaking only quantitatively different from healthy subjects.

At work equilibrium is maintained as long as the oxygen transport apparatus is capable of supplying the working muscles with sufficient amounts of oxygen, and muscular fatigue does not occur. When the muscles are not adequately supplied with oxygen, it is not possible to work for any length of time. Oxygen supply to the muscles is adequate as long as pulmonary ventilation still maintains a sufficient supply of oxygen to the blood and when at the same time cardiac output is such that sufficient amounts of oxygen can be brought to the muscles. The circulatory and pulmonary factors can limit oxygen supply independently of each other.

*It has been stated (1) that working capacity for practical use should be defined as the maximum working intensity consistent with steady state, (2) that the working intensity must be controlled by oxygen determinations if the type of work used for different persons does not give about the same efficiency, (3) that functions studied must be placed in relation to oxygen consumption, not to factors having no direct connection with working intensity, and (4) that the working intensity must be increased to, or nearly to, the maximum ability.*

The results obtained verify the assumption that bicycle ergometer work is a type of work well suited for examination of working capacity in a simple manner, as the working intensity can be used for approximation of the oxygen consumption. However, some subjects showed oxygen values that apparently were not adequate at higher loads, probably due to inability to increase the cardiac output. These subjects were too few to influence the over-all results to any extent. In this respect it may be considered more advantageous to replace oxygen consumption by working



intensity. The oxygen consumption values of the pulmonary groups were low but did not significantly differ from those of other groups (Tables 2 and 3). It is of course possible to use other types of work for working tests but then oxygen consumption must be determined for control of working intensity.

In contrast to the uniformity of oxygen consumption between the various groups, there appeared differences in pulse and respiratory reactions (Tables 7, 14 and 17). Pulmonary cases showed marked signs of the latter, whereas heart cases showed signs of both. In general the circulatory and pulmonary responses to work are found to be approximately parallel, except in some lung cases where respiratory reactions predominate (Tables 22 and 23).

*The results obtained show the importance of making working tests with varying and sufficiently high loads, and with reference both to circulatory and respiratory functions. Isolated factors such as vital capacity, residual air ratio, heart volume, blood pressure, or various electrocardiographic abnormalities do not necessarily give any information of the working capacity. For instance, an individual having a large heart may have an apparently normal or even hypernormal capacity. Nothing can, however, be said about future tendencies. One test does not give any information of a possibly declining working ability. It may consequently be necessary to make repeated tests for the proper understanding of the functional importance of clinical findings. This is especially obvious when considering patients newly recovered from rheumatic fever.*

There are at least three possibilities to explain why the clinical findings may not be correlated to working capacities. (a) The capacity is not in any way influenced by the »disease». (b) Those cases having low capacities but slight clinical signs may possibly have been influenced by the »disease», but may have had poor working capacities before the clinical signs appeared. (c) Cases with marked signs but with normal or hypernormal working capacity may before the beginning of the disease have had still higher capacities.

A working test can be used as a control of the subjective symptoms. When a person states that he cannot perform the slightest

work without breathlessness and palpitation, and nothing is found at the usual examination, the working test showing a capacity of at least 1200 kg-m/min, corresponding to an oxygen consumption greater than 2 liters per minute, something must be wrong — but apparently not with physical working capacity.

As discussed before it is impossible to speak of *one normal* working capacity. In this investigation 1200 kg-m/min was taken as the normal capacity, or rather, the basis for comparison. The capacities of those subjects in group 9 with leptosome body type seem to indicate that under special conditions 900 kg-m/min may be »normal». In addition the cases with slightly reduced working capacity in the normal groups 14 and 15 had low weight in relation to height. Generally there is a tendency to lower working capacity with decreasing body weight.

It can however be concluded that a working capacity of 1200 kg-m/min is high enough to exclude more severe heart or lung affections which may influence working capacity at the time of the examination. A working capacity in men of 600 kg-m/min is obviously abnormally low. For a capacity of 900 kg-m/min it is more difficult to draw definite conclusions. It may be necessary to consider body type, daily occupation, and whether the person has practised athletics or not.

*The usefulness of the working test employed can be summarized as follows.*

- (1) The working capacity can be estimated quantitatively, thus permitting guidance for occupational purposes or control of changes in the clinical state of a patient.*
- (2) The reliability of subjective symptoms can be controlled.*
- (3) Contribution to differentiation between relative heart and lung insufficiency.*

It is however not possible to draw definite conclusions of the cause of a reduced working capacity.

## SUMMARY

1. Earlier investigations on functional tests are briefly reviewed and criticized.
2. A standardized working test based on bicycle ergometer work with increasing loads is introduced.
3. The investigation covers patients with various diagnoses or symptoms of heart or lung diseases and ordinary healthy subjects as well as athletes.
4. Oxygen consumption and thus the mechanical efficiency of different individuals in bicycle ergometer work was shown to be fairly uniform.
5. Oxygen consumption in itself does not usually indicate the working capacity.
6. Ventilation of the lungs is shown to be unsuitable for determination of working capacity.
7. Pulse and respiratory rates are the most suitable indicators of working capacity.
8. The importance of determining working capacity for the proper understanding of clinical findings is demonstrated.

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# APPENDIX





# APPENDIX

## *Primary material.*

Key to table-headings.

### *Resting determinations.*

- a Age, years.
- b Body weight, kg.
- c Body height, cm.
- d Pulse rate.
- e Respiratory rate.
- f Vital capacity, liters.
- g  $\frac{\text{Residual air}}{\text{Total capacity}}$  %.

### *Determinations during work at 300, 600 etc. kg-m/min.*

- 1 Pulse rate after 6 minutes.
- 2 Respiratory rate.
- 3 Ventilation coefficient.
- 4 Oxygen consumption, centiliter/min.
  - 1) The subject is not working at an adequate rate.

### *Working capacity, work load divided by 100.*

- 5 According to pulse rate.
- 6 According to respiratory rate.

GROUP 1.

Case no.	a	b	c	d	e	f	600				900				1200				5	6
							1	2	3	4	1	2	3	4	1	2	3	4		
9	26	77	181	70		4.3	124	24.9	145	136		26.1	195	158		25.2	257	9		
26	21	66	168	80		3.2	136	19.8	141	168		22.3	176	184		23.9	229	6		
55	44	63	166	86		3.6	148	19.3	143	166		22.5	195	184		31.5	218	6		
89	26	75	182	90		4.9	130	20.7	152	166		20.9	201	192		24.7	254	9		
133	33	91	182	80		3.5	120	22.0	148	150		22.4	212	178		27.6	271	9		
136	23	76	174	72		3.0	126	17.7	153	154		18.6	210	174		20.9	262	6		
142	26	70	174	80		3.5	164	28.8	170	188		34.8	173					3		
144	32	85	186	68		3.9	126	21.6	146	158		22.0	203	186		27.8	281	9		
164	35	68	176	68		3.3	120	22.2	140	148		24.8	184					6		
169	31	65	171	102		4.0	150	19.2	132	172		19.8	180					6		
174	33	87	170	80		3.1	126	19.5	141	158				184		24.2	220	9		
178	23	83	176	68		4.0	124	15.5	143	150		16.4	194	176		19.1	238	9		
189	31	58	157	98	16	2.4	150	22	18.5	120	172	25	20.2	162	188	34	23.8	173	6	9
190	37	66	183	62	13	3.2	114	22	22.5	127	138	26	27.2	176	166	35	31.6	197	9	9
198	21	65	176	72	14	3.4	132	19	16.2	137	148	23	15.6	182	180	32	22.0	242	9	9
199	30			68	20	4.5	114	18	15.9	128	148	22	18.0	172	178	35	20.8	242	9	9

Case no.	300			
	1	2	3	4
55	128		19.3	99
142	136		29.8	104

GROUP 2.

Case no.	a	b	c	d	e	f	600				900				1200				5	6
							1	2	3	4	1	2	3	4	1	2	3	4		
75	22	75	189	80		5.7	112		17.8	142	134		19.4	196	150		20.7	249	15	
101	26	65	181	70		5.3	130		16.1	157	158		18.9	201	184		22.0	267	9	
122	27	69	182	64		3.9	114		34.2	127	150		32.4	181					6	
140	42	56	177	80		3.0	160		34.8	126									3	
163	36	75	164	82		3.8	142		16.7	151	170		17.8	212	184		22.6	254	6	
168	21	69	172	90		3.6	120		18.4	136	144		17.8	181	160		17.8	256	12	
186	21	66	167	74		4.0	128		17.3	138	160		18.9	196	188		22.2	207	6	
187	27	65	179	72	14	4.8	126	18	16.2	122	158	21	16.5	164	188	25	18.4	213	9	12
192	21	63	176	56	18	3.9	118	19	16.7	141	142	23	20.0	185	174	30	21.9	242	9	12
200	21	72	186	72	13	4.9	112	15	14.3	143	138	21	15.4	204	162	28	15.7	255	12	12
204	34	66	165	80	15	3.8	126	18			148	20	26.2	188	156	22			12	12

Case no.	300			
	1	3	4	
101	104	17.3	100	
140	134	27.6	85	

GROUP 3.

Case no.	a	b	c	d	e	f	600				900				1200			
							1	2	3	4	1	2	3	4	1	2	3	4
65	21	58	171	66		3.0	120		19.8	126	140		18.4	171	162		19.9	216
111	29	65	165	88		4.1	156		19.2	139	170		21.3	180	180		18.8	213
118	21	74	188	66		4.8	116		20.0	143	140		19.4	193	166		20.6	259
125	20	70	176	62		4.5	126		16.5	145	152		16.7	192	172		17.1	240
165	35	88	175	68		4.6	112		18.4	137	140		18.5	186	164		21.0	232
166	28	75	174	60		4.4	110		16.7	151	142		16.2	203	168		18.0	247
205	21	66	183	70	15	4.6	108	20	18.0	121	132		18.5	178	158	24	20.2	229
210	23	74	171	78	12	4.7	104		18.5	130	120		19.7	174	142	28	22.4	222

Case no.	300			
	1	3	4	
65	100	24.3	83	



GROUP 5.

Case no.	a	b	c	d	e	f	600				900				1200				5	6
							1	2	3	4	1	2	3	4	1	2	3	4		
5	41	68	163	108		3.6	174		20.8	153	192		23.8	192					3	
7	22	83	185	58		5.0	124		17.2	151	156		18.2	206	184		20.7	253	9	
47	25	71	178	108		3.5	168		23.4	150	188		24.9	198	188		25.6	230	3	
69	21	74	185	112		5.2	142		16.0	130	158		16.2	188	180		17.3	243	9	
107	27	73	181	56		4.8	104		15.8	139	124		16.1	190	160		17.4	245	12	
110	44	85	179	76		4.6	124		18.8	150	150		20.9	202	178		21.8	253	9	
112	37	75	175	86		3.8	146		23.2	154	164		24.2	204	194		26.4	252	6	
116	34	80	184	96		4.4	120		11.8	143	142		13.8	191	166		12.2	242	12	
129	42	103	182	64		4.3	102		18.9	143	132		19.8	198	164		21.5	241	9	
137	35	79	178	56		3.8	102		17.6	138	134		17.7	192	170		17.7	250	9	
138	22	74	177	70		3.5	142		18.1	124	168		18.8	180	190		20.3	245	9	
147	23	73	165	68		4.3	130		17.1	155	158		16.0	198	168		16.9	235	12	
151	33	95	182	88		4.0	120		21.4	153	156		24.6	220					6	
177	34	80	179	80		3.8	128		19.7	123	152		20.8	201	176		24.2	258	9	
193	46	73	171	82	14	4.0	140	26	25.7	150	172	35	28.6	212		39			6	
207	55	77	177	82	16	3.3	106	26			128	34			148				6	
221	38	69	179	80	20	4.0	116	20	21.4	136	150	25	23.0	191	168	30	27.2	243	12	
244	45	76	180	68	18	5.4	100	24			126	24			144	28	18.6	234	15	





# GROUP 6.

Case no.	a	b	c	d	e	f	600				900				1200				5	6
							1	2	3	4	1	2	3	4	1	2	3	4		
8	23	75	184	70		4.6	132		20.0	161	166		22.9	221	186		25.1	270	6	
10	20	74	186	64		4.4	148		13.2	151	174		13.8	196	200		15.3	249	6	
17	23	50	168	66		2.9	176		28.2	133	188								3	
23	28	64	172	68		4.6	124		18.5	138	148		16.6	198	184		17.7	260	9	
78	21	65	171	76		4.5	138		19.5	143	172		21.7	191	200		26.0	257	6	
88	21	77	175	68		4.3	122		19.3	155	156		20.0	204	170		22.4	243	9	
92	24	76	175	76		4.5	118		16.4	136	136		16.4	185	166		18.1	250	9	
93	47	74	177	72		3.7	102		21.7	132	114		22.0	171	128		21.8	212	18	
94	20	76	185	92		5.2	126		20.3	136	150		21.8	192	168		22.1	243	12	
96	21	70	182	104		4.5	172		20.3	153	184		20.5	207	200		22.8	246	3	
97	24	76	182	80		5.0	144		20.3	137	176		22.7	193	194		24.2	264	6	
99	21	66	176	66		3.7	126		16.9	135	162		18.2	176	182		19.2	224	9	
100	21	65	180	76		4.5	158		19.8	143	180		20.4	187	194		22.8	243	6	
104	32	50	169	78		3.5	136		22.2	136	160		22.8	187	180		23.3	231	9	
106	21	74	173	66		4.3	108		18.6	134	128		20.3	172	152		20.0	241	12	
115	21	84	180	58		4.5	102		16.9	141	116		17.2	181	130		18.0	214	18	
119	23	57	166	82		3.4	142		21.3	130	172		23.3	185					6	
121	19	80	188	70		5.1	110		18.4	128	132		20.3	183	156		20.6	244	12	



# GROUP 7.

Case no.	a	b	c	d	e	f.	g	300				600				900				1200				5	6
								1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
4	28	74	167	76		4.7		116		19.8	106	132		19.4	157	158	19.2	210	182			21.4	252	9	
6	23	78	183	74		4.1						134		17.6	154	148	17.0	195	168			16.4	243	12	
29	23	61	169	70		3.0		126				126		21.2	143	150	20.6	193	186			19.1	244	9	
43	29	70	168	74		3.7		124				124		19.1	141	150	18.0	191	166			20.0	245	12	
64	28	70	171	42		4.4		92				92		18.7	151	110	20.5	199	136			22.0	265	15	
91	20	75	174	74		4.2		118				118		18.4	143	148	22.2	198	176			19.2	242	9	
98	21	85	179	52		4.7		116				116		13.8	143	146	14.9	196	174			15.4	247	9	
103	34	82	192	68		5.0		106				106		17.1	131	124	17.1	179	152			18.5	231	12	
113	23	62	166	82		4.0		140		19.4	101	140		16.2	146	172	18.0	200	190			20.6	256	6	
117	25	68	175	60		5.0		126				126		17.9	145	144	19.4	182	166			20.2	239	9	
150	22	60	161	48		3.4		118				118		17.6	144	152	17.2	186	182			19.3	245	9	
157	24	65	176	64		4.5								17.0	130	122	17.6	193	140			17.6	210	15	
183	22	82	179	72	14	4.6		120	18			120	18	19.6	153	138	20	18.2	193		23	19.4	244	12	12
208	20	83	177	48	17	4.7		100	24			100	24	18.9	152	122	24	19.4	194		28	19.4	241	15	12
224	43	70	170	58	18	4.2		118	28			118	28			132	28							15	?
227	57	90	175	68	14	4.3		80	20	18.7	109	96	14	18.0	146	118	16	19.1	185		21	20.9	228	12	12
258	39	64	173	60	12	4.4		88	13	18.1	100	106	15	17.2	150	126	16		138		18			15	12
275	42	70	167	52	14	4.5		84	20	18.0	105	108				130	24	18.4	202		27	20.0	258	12	12
277	51	106	179	54	15	3.5		88	18	19.6	115	104	21	18.9	169	126	25	20.2	212		28	24.1	266	12	12
285	49	82	170	50	16	4.1		84	20	20.3	103	98	21	20.2	128	124	21	22.1	188		30	24.1	235	12	12
290	46	79	180	64	19	5.5		84	18	16.9	110	96	19	16.9	144	124	20	16.2	198		22	17.6	254	12	12



# GROUP 8.

Case no.	a	b	c	d	e	f	600				900				1200				5	6
							600				900				1200					
							1	2	3	4	1	2	3	4	1	2	3	4		
32	24	73	172	70		3.4	116	19.8	143	136	18.8	194	148	20.0	229	15				
45	41	78	177	92		3.7	130	20.0	141	154	24.0	215	176	28.8	277	9				
50	39	108	171	92		3.3	124	23.5	166	142	27.8	217	166	19.6	225	9				
52	22	62	176	58		4.2	104	16.9	124	132	19.2	172	194	22.5	234	9				
60	20	68	173	82		4.6	144	17.6	128	172	20.7	185	152	21.4	242	6				
63	23	75	189	74		5.8	120	20.1	144	132	20.7	186	140	17.7	220	12				
70	31	66	177	70		4.8	108	16.4	122	122	16.9	170	170	20.2	240	15				
71	27	69	179	56		4.8	118	15.8	142	142	18.9	193	170	20.2	240	12				
72	31	69	175	58		4.2	100	20.7	130	114	21.1	177	140	21.2	231	15				
85	32	74	187	60		4.9	110	18.0	141	134	18.5	186	152	19.7	232	12				
105	30	75	176	84		4.3	132	16.1	145	166	18.7	193	180	17.3	270	9				
124	23	75	188	78		4.8	122	20.1	138	142	20.9	201	166	22.4	262	12				
134	23	70	182	52		5.0	98	17.6	138	122	18.0	191	148	16.8	251	12				
155	31	75	181	56		5.3	100	18.7	142	114			138	20.1	248	15				
171	47	74	172	80		3.9	114	25.9	143					30.2	251	(3)				
173	25	69	189	86		5.2	146	22.8	133	178	23.1	191	200	18.9	230	6				
181	23	73	179	80		4.4	124	18.1	128	142	17.4	181	156	25	21.0	12				
184	23	73	179	56	15	4.2	112	19	18.1	129	18.5	176	172	25	21.0	9	12			



# GROUP 9.

Case no.	a	b	c	d	e	f	600				900				1200				5	6
							1	2	3	4	1	2	3	4	1	2	3	4		
2	27	75	173	106		4.2	148	21.3	143	158		22.7	202	168					12	
3	30	60	170	80		3.8	156	19.4	124	168		19.4	184				23.8	250	9	
13	24	65	175	82		4.6	136	18.3	153	164		20.4	198	188			18.4	232	9	
14	29	65	172	78		3.9	110	18.8	134	132		17.6	186	154			23.5	255	12	
15	21	78	182	94		4.8	140	20.3	156	166		22.6	203	184			16.7	231	9	
16	32	79	177	90		3.6	124	16.6	132	146		16.4	173	158			27.7	205	12	
18	21	57	171	62		3.1	154	24.3	158	172		26.6	196	184					6	
24	21	71	183	90		3.4	146	17.6	153	164		18.6	201						9	
25	22	72	182	88		4.3	136			144		22.4	189	156			25.0	223	15	
28	23	75	183	76		4.3	124	17.9	148	148		18.2	192	170			16.5	264	9	
30	21	61	167	70		4.0	140	19.1	148	156				<sup>1)</sup> 162					12	
31	21	66	174	78		3.4	134	18.9	142	160		21.4	192	<sup>1)</sup> 162					9	
33	28	76	175	104		3.1	142	22.3	156	162		23.6	202	180			25.8	250	9	
34	21	68	175	56		4.2	116	22.1	141	136		21.6	178	156			24.2	221	12	
36	23	60	176	98		3.7	144	19.7	142	174		21.8	188	198			24.3	237	6	
38	29	71	183	92		5.5	132	15.9	139	172		16.2	196	184			17.8	245	6	
40	21	65	183	84		3.8	124	20.1	146	144		24.8	190	174			26.2	241	9	
41	36	82	171	78		4.3	140			152		21.2	204	164			22.3	249	12	
42	42	74	174	86		4.1	120	17.8	143	132		16.0	184	150			16.7	237	15	
44	44	86	175	74		4.4	108	23.0	152	120				136					18	
46	23	55	176	76		4.7	138	21.0	137	172		24.0	192	180			28.1	216	6	
48	20	62	177	108		3.3	160	25.1	138	180		28.5	185						6	
49	23	65	178	64		4.3	122	18.4	149	140		19.1	196	162			19.9	248	12	
51	21	75	174	64		3.9	116	23.5	133	144		24.3	182	176			27.5	229	9	
53	22	77	180	86		5.0	132	40.7	130	142				152			27.1	237	15	





# GROUP 9. (Continued)

Case no.	600						f	c	d	e	900						1200						5	6
	a	b	c	d	e	f					1	2	3	4	1	2	3	4	1	2	3	4		
176	29	75	176	66		5.2	122				137	148	17.1	186	172		18.7	224					9	
179	30	57	176	68		3.6	130				123	156	18.6	177									9	
182	22	70	175	120		5.4	148				134	160	21.3	178	184		22.8	215					9	
291	46	59	168	56	17	4.0	90				131	116	24	19.5	179		32	20.5	237				12	9
344	37	69	167	66	14	5.0	98				122	122	17	14.3	165		18	15.7	210				12	12
355	27	59	166	60	16	4.6	106				124	134	18	15.6	181		24	17.0	232				9	12
358	31	82	179	58	19	5.1	110				136	136	27	17.1	195		32	19.4	261				12	9
369	26	68	177	72	18	5.0	128				138	154	24	17.2	197		28	17.0	252				9	12
373	29	78	183	78	16	5.1	116				136	138	26	17.5	191		30	17.7	244				12	12
374	48	82	162	80	20	3.7	116				157	134	26	19.6	212		30	21.9	267				15	12
375	45	74	170	60	19	4.6	96				139	126	32	25.5	182		40	22.2	236				12	6
379	32	87	180	60	23	5.1	118				141	154	27	18.7	197		24	20.6	238				12	12
387	38	56	166	74	19	3.5	114				132	132	26	16.8	175		33	17.3	224				15	9

Case no.	300				Case no.				300			
	1	2	3	4	1	2	3	4	1	2	3	4
2	138		23.2	102	41	124		108			18.9	
3	128				42	106		101			15.4	
15	110		19.1	106	46	94		104			25.4	
16	112		17.0	99	53	116		95			39.0	
25	124		28.5	100	291	78		90			19.3	



GROUP 10. (Continued)

Case no.	a	b	c	d	e	f	g	300				600				900				1200				5	6
								1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
284	50	58	174	62	17	3.1	43	108	22	19.2	106	128	28	19.7	139	152	30	19.0	190	172	34	22.8	227	9	9
287	53	74	168	50	16	4.3	34					98	20	17.9	139	128	28	21.0	186	162	33	22.2	254	12	9
295	58	107	179	78	17	3.9	34	92	19	12.8	106	104	22	19.3	142	114	24	21.6	186	128	28	21.3	232	21	12
299	52	92	176	74	12	4.7	34	110	10	15.3	116	132	10	16.8	164	154	15	17.3	211	174	24	21.6	262	9	12
309	41	79	177	58	23	4.1	38					100	37	24.4	135	114	40	25.4	185	134	41	21.4	227	18	3
310	50	71	178	82	14	4.4	31					104	18	19.4	123	130	23	21.0	176	148	28	22.4	249	12	12
315	51	61	166	62	18	4.6	35					114	23	20.8	139	140	27	22.1	179	170	37	27.9	243	9	9
322		77	176	78	15	4.3	36					102	16	20.7	124	128	21	21.3	173	160	25	24.1	228	12	12
345	39	81	174	64	15	5.0	34					98	16	16.4	131	116	20	16.8	184	152	25	19.5	249	12	12
378	47	66	170	58	19	3.8	36	98	26	17.8	106	114	30	18.3	141	136	31	16.9	183	160	32	17.4	220	12	6
380	52	96	178	90	18	4.6	38					120	21	17.1	146	132	24	16.7	181	144	26	17.0	228	18	12
413	64	76	170	70	20	4.5	32	116	19	17.3	110	136	21	19.0	147	154	24	21.3	183	176	29	21.5	250	9	12

GROUP 11.

Case no.	a	b	c	d	e	f	g	600				900				1200				5	6
								1	2	3	4	1	2	3	4	1	2	3	4		
245	47	62	173	74	20	4.1	32	142	22			158	23	17.0	177	170	24	16.8	229	12	12
248	32	76	181	62	15	5.4	30	108	17	14.7	135	130	18	15.7	175	154	20	15.2	225	12	12
261	66	85	164	60	15	4.4	31	114	18	19.0	151	144	22	21.8	201	<sup>1)</sup> 158	<sup>1)</sup> 31			9	9
262	54	56	170	62	15	3.7	31	120	17	19.1	127	146	22	19.6	172	166	26	20.9	220	12	12
306	56	69	181	62	15	5.5	31	100	19			128	21	17.2	189	158	22	20.0	240	12	12
317	48	62	170	68	16	3.6	30	106	22	17.7	122	140	24	17.3	182	164	28	17.9	238	12	12
318	37	74	183	76	18	4.2	31	106	19	16.4	152	126	21	17.8	189	148	24	19.5	231	15	12
326	37	61	166	68	26	3.4	32	<sup>1)</sup> 118	<sup>1)</sup> 30	<sup>1)</sup> 19.6	<sup>1)</sup> 114	<sup>1)</sup> 134	<sup>1)</sup> 35	<sup>1)</sup> 21.1	<sup>1)</sup> 132	<sup>1)</sup> 144	<sup>1)</sup> 36	<sup>1)</sup> 20.0	<sup>1)</sup> 149	9	6
334	37	68	181	74	19	5.1	31	<sup>1)</sup> 116	<sup>1)</sup> 16	<sup>1)</sup> 17.3	<sup>1)</sup> 126	<sup>1)</sup> 134	<sup>1)</sup> 17	<sup>1)</sup> 16.1	<sup>1)</sup> 168	<sup>1)</sup> 144	<sup>1)</sup> 19			15	12
341	44	71	167	72	15	3.3	32	116	14	15.2	121	140	17	15.7	183	162	22	18.6	236	12	12
363	51	71	190	78	22	5.1	32	112	26	20.0	122	146	20	19.2	171	164	26	19.6	226	12	12
371	55	64	173	58	18	4.1	33	98	20	17.0	135	122	21	16.4	184					12	?
383	55	87	177	70	17	4.0	32	104	26	19.7	129	128	25	20.1	217	152	36	23.4	264	12	9
390	55	63	168	60	17	3.8	33	104	18			130	21			140	26			15	12
394	49	52	167	62	15	3.9	31	100	18	16.3	127	126	22	16.2	174	142	29	18.6	222	15	12
409	48	63	174	54	16	4.5	33	128	17			160	21	16.2	178	<sup>1)</sup> 172	<sup>1)</sup> 31	<sup>1)</sup> 17.8	<sup>1)</sup> 217	9	9

Case no.	300			
	1	2	3	4
245	128	22		
248	96	16	14.4	100
261	100	17	17.8	97
262	104	14	19.3	91

GROUP 12.

Case no.	a	b	c	d	e	f	g	300				600				900				1200				5	6
								1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
229	53	75	179	62	17	6.0	35	94	20	21.4	95	110	22	20.0	126	148	23	20.7	175	172	25	22.4	227	9	12
253	60	84	181	60		3.5	40	80	17	22.5	94	94	19	18.1	143	112	20	21.3	151					18	12?
255	43	62	167	60	15	3.3	38	92	16	17.2	94	108	18			118	20			126	19.4	198	21	12?	
280	58	66	167	72	18	3.3	49	98	23	22.2	100	112	24	23.9	131	128	26	27.7	177	138	36	31.6	213	18	9
288	61	60		50	14	3.9	38	80	15	17.6	92	94	24	19.8	127	112	25	19.9	165	132	27	21.3	193	15	12?
293	56	53	171	60	17	2.9	99	94	20			118	22	20.0	199	160	26	20.4	125	170	30			9	9
296	40	74	182	58	14	5.0	34	82	18	15.9	99	96	19	14.9	132	118	20	16.3	178	146	21	16.7	229	15	12
298	49	75	169	76	16	4.1	36	96	22	23.2	102	114	24	20.1	148	138	26	21.1	185	146	29	23.6	221	15	12
319	46	68	168	82	17	3.2	49					140	21	17.0	124	160	24	16.8	178	172	31			9	9
321	44	83	179	58	15	4.9	34					92	17	20.4	133	118	20	20.2	198	138	22	21.5	241	15	12
328	40	82	177	70	14	4.1	37					106	21	18.4	137	126	24	18.8	183	150	25	19.8	239	12	12
331	55	79	180	60	19	3.0	38	100	20	21.0	86	124	23	20.3	119	150	25	23.2	160	160	33			9	9
335	43	79	175	60	16	4.1	37					104	15	15.9	128	118	21	17.8	171	140	25	19.6	229	15	12
338	56	69	172	60	13	4.1	47					108	18	18.3	128	138	21	19.3	171	168	25	22.0	228	9	12
352	58	60	166	62	22	3.4	43	94	25	20.6	92	108	25	17.7	119	116	26	18.3	132				15	?	
360	39	54	165	76	23	3.5	35					128	25	18.7	122	154	28	18.4	180	172	37	16.8	243	9	9
364	29	70	174	78	19	3.9	34					106	28	21.4	120	126	32	19.9	158	150	34	19.5	209	12	6
385	53	68	171	46	14	4.9	35					88	17	15.1	120	112	20	16.2	189	134	24	17.7	236	15	12
402	46	72	173	60	15	2.8	42					114	17	18.2	125	158	21	18.9	147	176	28	21.0	177	6	9?
405	46	56	165	62	17	3.2	37					112	19	21.0	125	142	25	22.0	173	166	31	24.6	226	12	9

GROUP 13.

Case no.	a	b	c	d	e	f	g	300				600				900				1200				5	6
								1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
219	48	89	171	64		3.0		92	19	20.2	89	114		23.5	139	140		24.8	201					9	
222	58	64	169	64	16	4.6		98		21.0	88	138	20	24.7	132	164	22	26.2	147					9	?
226	34	83	179	60	21	5.0		94	20	18.6	93	122	27	21.4	143	140	30				32			12	9
232	41	79	183	64	13	5.4		94	17	16.6	100	114	17	16.7	137	142	21	18.2	186		22	18.4	236	12	12
233	46	81	176	46	18	4.6		72	20	23.2	95	92	22	19.6	140	118	30	21.8	197		35	24.8	255	12	9
234	45	69	168	62	18	4.5		92	24	21.5	97	106	28	21.7	139	146	36	23.4	187		38	28.6	227	9	6
235	47	63	172	62	17	5.3		98	19	17.2	102	118	19			146	20				27	21.3	236	12	12
236	53	77	164	60	17	4.0		88	20	20.5	98	116	23	21.3	132	150	26	22.2	195		28	24.6	242	9	12
241	36	49	168	52	16	3.6		92				118				148	30			174	39			9	9
242	33	72	178	54	15	4.6		78	16			94	17			116	18	16.5	193		18	16.5	245	12	12
246	46	67	170	78	15	3.8		110	18	21.9	87	128	20	21.7	122	152	27	23.6	181		32	24.8	226	9	9
249	41	67	167	60	18	4.9		92	20	19.5	90	110	20	15.3	143	132	21	18.4	187		24	21.1	233	12	12
250	39	101	180	62	11	5.3		84	12	17.1	110	100	12	15.9	149	122	13	16.3	193		16	16.9	245	12	12
251	41	57	165	70	20	5.0		98	26	21.3	80	122	24	19.6	120	138	28	19.1	161		30	20.9	218	12	12
254	33	91	190	58	18	6.8		84	19			102	20	15.6	142	118	20	14.7	183		21	16.1	237	15	12
257	36	66	169	72	14	5.0		104	14	15.2	102	124	15	15.5	138	154	16	15.4	178		23	18.2	231	9	12
264	41	70	181	58	15	5.6		90	18			104	20			136	21				23	18.0	235	12	12
265	34	76	175	68	19	5.2		104	24	20.0	111	118	26	20.0	147	140	28	20.1	199		36	22.3	268	9	9
266	47	71	172	80	18	5.0						118	17	18.0	143	144	18	16.8	203		20	19.6	260	9	12
267	46	64	172	62	14	4.6						102	22	16.8	133	118	25	17.8	181		27	19.4	238	12	12
268	35	81	184	54	17	5.5						92	21	22.4	139	112	29	20.4	192		30	20.5	243	15	12
270	37	68	177	52	15	4.9						102	17	16.9	138	134	19	16.9	196		23	18.0	238	9	12
278	40	87	180	80	15	4.1		108	15	20.0	105	124	18	20.2	143	148	22	22.8	186		26	25.3	202	12	12
279	41	68	185	82	13	5.1		112	12	19.0	99	128	14	18.1	143	142	15	17.5	180		20	18.3	222	15	12
282	35	69	177	54	13	4.7						98	16	20.1	128	124	21	19.3	186		24	20.3	232	12	12

# GROUP 13 (Continued)

Case no.	a	b	c	d	e	f	g	300				600				900				1200				5	6
								1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
286	41	72	172	62	14	5.1		100	22	15.7	105	94	18	18.1	127	116	21	17.7	180	146	24	19.4	234	15	12
292	40	79	170	66	17	4.4		90	15	15.3	105	116	20	16.2	133	130	22	15.4	176	150	24	16.5	222	12	12
294	40	82	176	62	16	4.5		90	23	19.1	103	112	23	15.6	146	134	26	16.8	202	158	28	18.0	270	12	12
297	41	81	179	66	16	6.0		90	23	19.1	103	106	16	14.8	142	126	17	15.3	180	148	18	15.8	233	15	12
300	52	96	169	70	21	3.9		90	23	19.1	103	100	25	19.4	148	124	40	20.3	200	150	36	22.9	246	12	6
301	38	80	181	76	23	4.5		142	32	19.3	106	154	32	18.2	137	174	28	16.1	185	186	31	18.7	229	6	9
303	42	94	177	80	13	5.2						110	20	18.4	149	128	21	18.6	188	152	26	20.0	237	12	12
305	38	70	161	70	16	3.2						116	21	20.0	131	136	22			160	32	23.6	248	12	9
307	42	88	180	64	16	5.8						100	16	16.1	132	126	17	18.8	181	148	18	19.5	240	12	12
308	40	67	170	54	17	4.6						100	21	17.9	139	116	22	18.1	187	140	26	19.2	235	15	12
311	45	69	178	72	15	6.0						106	18	14.3	133	140	19	14.3	193	176	20	17.0	254	9	12
312	38	81	172	58	20	4.7						106	27	19.9	137	134	28	20.0	181	154	29	20.9	230	12	12
313	31	78	177	70	15	5.0						96	16	13.8	134	112	18	14.9	184	144	22	17.2	237	12	12
314	46	82	169	82	13	4.2						124	20	18.3	142	150	22	18.2	190	174	25	20.0	245	9	12
316	33	59	159	64	20	3.7						122	23	20.3	123	176	32	21.8	185	184	37	25.2	232	6	6
324	36	62	168	54	17	4.5						106	20	15.8	138	130	22	15.0	182	152	23	17.8	245	12	12
330	29	70	185	62	18	5.5						96	20	16.7	131	120	21	16.3	181	146	26	16.0	228	12	12
332	32	70	185	58	21	4.8						106	20	17.2	135	128	25	18.8	193	140	36	19.5	220	12	9
333	35	68	173	66	15	5.2						106	16	18.0	134	130	21	18.7	180	148	24	19.8	211	15	12
337	35	71	173	62	17	4.8						92	19	15.9	126	124	21	14.2	186	164	24	15.6	222	12	12
339	33	70	172	64	20	5.1						124	22	18.4	126	162	25	21.8	187	186	31	27.2	241	9	9
342	47	68	170	68	14	4.4						114	21	19.3	122	140	24	22.2	172	174	35	28.5	235	9	9
343	40	80	176	80	19	4.3						128	21	16.0	129	168	25	17.5	184	196	30	20.0	218	9	12
346	37	61	180	62	20	4.3						110	27	22.4	122	128	28	20.5	181	154	29	21.9	232	12	12
349	45	56	179	64	20	4.3						106	25	19.0	125	142	28	20.5	182	164	38	25.6	214	9	9

350	37	83	172	66	18	4.6
354	26	69	171	66	16	4.7
359	35	65	171	70	15	5.1
368	28	71	183	72	17	6.4
370	35	69	173	62	19	5.0
376	30	70	178	50	16	5.6
377	37	76	171	66	17	5.1
382	50	78	185	50	16	5.5
384	43	68	176	50	14	5.4
388	50	87	179	68	18	5.0
393	32	66	172	54	17	5.9
398	41	62	176	62	20	5.0
400	28	73	175	86	13	4.2
403	46	99	178	66	16	5.2
404	42	76	170	62	17	3.8
406	41	80	165	60	17	4.5
407	47	68	164	62	12	4.5



# GROUP 14.

Case no.	a	b	c	d	e	f	600				900				1200				5	6
							1	2	3	4	1	2	3	4	1	2	3	4		
11	45	75	173	86		3.5	122		19.8	148	146		20.8	185	156		22.2	212	12	
19	21	60	180	66		3.3	136		23.5	141	152		20.8	195	178				9	
20	21	62	176	72		3.9	140		20.7	146	154		19.4	195	168		21.4	235	12	
21	39	76	178	60		4.3	114				132		22.8	192	144		20.6	248	12	
37	23	70	168	84		4.0	118		15.3	151	132		15.0	183	150		16.3	224	15	
57	37	77	169	62		3.1	114		25.0	135	124		23.7	190	148		22.6	243	12	
73	30	62	177	68		5.0	110		15.6	130	128				158		18.4	222	12	
79	20	74	182	90		3.6	126		18.3	144	144		20.0	191	152		20.0	235	12	
80	21	68	169	94		4.5	130		15.1	150	152		15.6	196	166		15.9	248	12	
86	36	68	175	60	13	4.2	116	15			134	19			156	24			12	12
128	22	79	185	56	14	5.7	94	13			124	16			148	17			12	12
132	60	69	171	72	15	2.9	124	19			150	24			176	32			9	9
260	44	69	171	58	16	4.2	100	18	17.1	130	120	21			150	24	19.6	232	12	12
263	24	87	182	58	12	6.0	102	13	13.8	144	136	14	14.2	189	154	16	15.0	243	12	12
289	26	67	174	60	16	4.4	102	22	19.6	126	130	26	21.5	172	158	32	24.6	241	12	9
320	41	77	177	66	12	5.7	90	14	17.6	134	112	16	18.4	177	148	19	21.0	259	12	12
323	32	64	172	60	13	4.7	104	18	17.1	127	114	21	18.3	168	140	24	19.6	212	15	12
340	39	73	170	60	11	5.0	100	12	14.4	133	124	14	15.4	168	162	16	17.7	229	12	12
347	33	79	175	58	14	5.1	84	18	16.3	137	96	19	16.9	188	114	21	17.7	248	21	12
348	28	60	169	58	15	4.2	112	18	18.1	142	136	21	18.0	182	164	26	21.0	243	12	12
361	26	70	178	60		4.3	106		17.9	145	124		18.4	185	144		19.3	230	15	
362	26	73	173	82		4.5	128		16.0	137	152		17.9	191	170		18.8	235	12	
366	40	64	158	68	17	2.9	98	20	17.4	123	128	22	17.3	184	156	24	19.0	220	12	12
381	43	101	183	66	20	5.3	104	19	17.8	138	122	21	16.5	193	144	22	17.5	250	15	12
386	31	62	168	66	16	5.0	118	17	15.3	134	136	20	15.6	191	158	22	17.0	239	12	12

389	50	69	166	84	19	4.8	126	26	16.1	152	30	16.7	198	160	35	19.3	246	12	9
391	37	89	181	58	14	5.0	136	19	13.2	152	20	13.8	184	166	22	15.8	242	12	12
392	28	70	177	66	15	4.4	106	14	19.1	130	16	16.8	185	150	16	19.3	231	12	12
410	30	80	174	62	16	5.6	118	16	18.9	121	19	20.3	189	166	24	23.1	221	12	12
412	46	72	176	70	15	4.4	120	15	17.5	146	20	18.1	195	166	24	19.6	242	12	12
414	24	74	174	64			118		16.1	141		16.6	196	164		18.1	254	9	
415	24	63	173	70		4.4	122		15.4	143		16.1	183	174		17.7	233	12	12
416	35	70	175	56		4.4	110		16.3	130		19.0	189	166		18.2	247	12	12
417	20	72	181	68		4.6	118		19.4	121		19.2	188	162		19.7	238	12	12
418	27	74	176	60			116			134				152				12	12
419	34	60	162	88	12	4.8	126	15		148	20			166	24			12	12
420	29	71	181	80	16	4.7	134	17		154	21			168	26			12	12
421	26	67	186	92	19	3.1	134	21		162	26			182	31			9	9
422	24	82	186	86	20		134	20		156	22			174	26			9	12
423	35	67	173	66	17	4.4	122	20		142	23			162	25			12	12

Case no.	300				1500			
	1	2	3	4	1	2	3	4
21	92		25.4	96				
128					166	20		
260	84	16	18.3	94				
263	90	12	13.8	100				
361					168		24.9	284
362					192		20.0	281
418					170		21.0	281
420					184	33		
421					196	34		
422					184	29		
423					178	31		



GROUP 16.

Case no.	a	b	c	d	e	f	g	600				900				1200				1500				5	6
								1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
1	20	66	171	52	15			94	21			110	23			122	26			140	28			18	15
123	22			74	14			108	14			120	16			134	17			150	19			18	18
141	22	70	181	52		4.8		102		16.0	143	120		15.9	185	138		15.4	154			15.7	296	15	
202	29	68	172	50		4.8		94		16.6	140	114		16.4	185	146		15.6	164					15	
209	25	85	182	60	12	6.3		98	16	15.5	137	112	17	16.5	180	128	18	14.9	142	19		16.0	295	18	15
218	24	67	177	58	13	4.7		124	16	15.3	148	146	17	14.6	198	168	24	16.8						12	12
395	41			58	16			100	20			120	23			146	26			168	33			15	12
450	19	64	174	64		4.3		120		16.3	134	136		17.3	182	158	20	17.9	164					12	18
451	20	67	175	70	12	4.9		112	15			126	17			148				25				15	
452	22	76	172	68		5.4						122		17.5	179	152		16.6	170			17.5	288	15	
453	21	76	189	60		5.0						122		15.9	183	132		15.8	140			16.6	294	24	
454	25	69	186	76		6.0						142		18.8	179	160		21.6	194			26.8	288	12	
455	22	72	174	60		4.5						118		15.9	181	150		16.0	170			16.5	293	15	
456	24	60	173	60		4.5						120		14.7	199	146		15.1	164			15.9	312	15	
457	23	75	173	48		4.3		82		17.9	134	96				112		19.6						21	
458	20	75	184	56		5.0		94		18.1	146	114		18.6	197	124		19.9						21	
459	27	64	173	44		4.5						88				104			120					27	
460	20	68	181	68				108				126		15.9	188	150		16.0	172			15.2	307	12	







# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CCXVIa (216a)

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## CLEARANCE TESTS IN RENAL DISORDERS AND HYPERTENSION

BY

*OLLE HOGEMAN*

ACCOMPANIES VOL. CXXXII (132)

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Translated by Erica Odelberg

FROM THE MEDICAL CLINIC (HEAD: PROFESSOR ERIK ASK-UPMARK, M. D.)  
AND THE CENTRAL LABORATORY (HEAD: LABORATOR ANDERS GRÖNWALL, M. D.)  
OF THE UNIVERSITY OF UPSALA

AND

THE STATE INSTITUTE OF HUMAN GENETICS AND RACE BIOLOGY, UPSALA  
(HEAD: PROFESSOR GUNNAR DAHLBERG, M. D.)

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CLEARANCE TESTS  
IN  
RENAL DISORDERS AND  
HYPERTENSION

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UPPSALA 1948  
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## Preface

When the present investigation was started, clearance tests were relatively unknown in clinical practice. The work was made more difficult and retarded by the conditions of war, since among other disadvantages, satisfactory reagents could not always be obtained. Now that the results can finally be published, these tests are still far too little known and the writer therefore made a closer analysis of their clinical use in order to contribute to our knowledge of them.

Thus the present investigation, started in the autumn of 1941, is a survey of the results achieved by the writer in the study of some of the most common renal disorders within the scope of internal medicine. In addition to actual renal diseases, essential hypertension — with and without symptoms from the kidneys — has been included in order to throw some light on the diagnostic and prognostic possibilities afforded by the clearance tests.

During the course of the work it appeared to be of special interest to make some study of the reserve capacity of the kidney and of those questions correlated with the regulation mechanism in damaged kidneys. The writer therefore investigated a small number of cases with *one* functioning kidney, due either to nephrectomy or to some other cause.

In order to be able to complete the investigations, I applied for an appointment at the Medical Clinic of the University Hospital in Upsala, and have been working there since 1944. The material on which the investigation is based is collected entirely from this Clinic during the years 1942—1948. From 1942 to 1946, the Physician-in-Chief was Professor Gustav Bergmark, my clinical teacher during my medical studies and thereafter my first Chief. From every point of view my

debt of gratitude to him is considerable. He has been not only the model clinician and teacher, ever ready to help and guide the inexperienced, but also — and often unobtrusively — he has smoothed my path and encouraged my work in every way. For all this and for his faithful and unfailing friendship I wish to thank him.

My warm thanks are also due to my present Chief, Professor Erik Ask-Upmark. As Head of the Clinic since 1946, when the greater part of the elinical work was already accomplished, he has facilitated its completion in every way. Owing to his extensive knowledge of the diseases treated, to which he himself has devoted much interest, my discussions with him have been extremely profitable. I also wish to thank him for the years during which I have had the advantage of being his assistant at the Clinic.

It is my pleasant duty to express my thanks to Professor Jan Waldenström, Head of the Institute of Theoretical Medicine in Upsala, for his long-standing friendship and for all the valuable discussions due to his incentive. He has always shown great interest in my problems and taken time to appreciate them.

For the statistical treatment of the material I turned to the Swedish State Institute of Human Genetics and Race Biology and to its Chief, Professor Gunnar Dahlberg. Notwithstanding his considerable daily burden of work, he devoted himself to my work and my problems in a manner which have earned my deepest gratitude. He has given me much valuable advice regarding the disposition and the planning of the paper. His interest has always been vital and personal and my appeals for help have never gone unanswered. I am deeply indebted to him for his generous assistance and for many inspiring discussions.

I wish to thank Professor Gunnar Blix for allowing me to work at the Institute of Medical Chemistry and for all his valuable advice and encouragement in elaborating the technique for the iodine determinations. My thanks are also due to Professor Torsten Teorell for discussions concerning certain physiological matters.

I wish to convey my thanks to my friend Laborator Anders Grönwall, M.D., for his great interest and help during the investigation. During my work in the Central Laboratory he has placed all its resources at my disposal and facilitated my work in every way. I also wish to thank him for his personal interest and for many profitable discussions.

The Misses Mirjam Brännström and Gerd Ericson, laboratory nurses, have carried out the main part of the chemical analyses and had charge of the practical performance of the clearance tests. The statistical calculations were carried out mainly by Mrs. Martha Bume and Mr. Bo Kjessel. They all performed their work in an exemplary and accurate manner and I tender my thanks for their valuable assistance.

I wish to thank Mrs. Erica Odelberg who translated the paper into English with care and rapidity.

Grateful acknowledgement is also made for financial grants from the following institutions: The Swedish Society for Medical Research, the Foundation »Thérèse och Johan Anderssons Minne», King Gustaf V's Jubilee Fund, the Regnell Foundation, the Scandinavian Insulin Foundation and the Swedish State Funds for Promoting Medical Research.

Last but not least I wish to thank Consul-General Gunnar Carlsson, Gothenburg, who during the most difficult war years helped a physician entirely unknown to him to obtain certain reagents by means of convoy boats. Without these my work would have been interrupted for several years.

Upsala, October 1948.

*Olle Hogeman.*



## CHAPTER I.

# SURVEY OF THE LITERATURE

### Normal Renal Function

The quantitative measurement of renal function has only comparatively recently been studied.

The nephron was realized to be a complete unit only when Bowman in 1842 discovered that the glomeruli are in direct connexion with the tubules and investigated the blood supply from the former to the latter. Bowman's theory concerning renal function, later elaborated by Heidenhain (1874) assumed a secretion of the majority of the components of urine from the tubules, whereas water and a number of salts, e.g. sodium chloride, are secreted in the glomeruli. In opposition to this so-called »vitalistic» theory we have that expressed by Ludwig (1844) and later by Cushny (1917) and Rehberg (1926) of filtration and reabsorption. Ludwig already put forward the hypothesis that a simple filtration of plasma together with the substances dissolved in it took place in the glomeruli, whereas the protein was retained and the final urine formed in the tubules by the majority of the filtrate being reabsorbed by simple rediffusion. Cushny's and Rehberg's investigations afford proof that this latter process is not so simple but actually involves both diffusion of certain substances such as urea, and active reabsorption of others, as for example chloride, glucose and in general those designated with the term »threshold substances». Furthermore, it has been shown through later investigations by American workers that an active secretion from the tubular cells takes place under certain conditions

(Elsom, Bott and Shiels 1936, Shannon 1935, Smith 1937, and others).

Our present conception of renal function can be summarized briefly as follows: a) ultrafiltration in the glomeruli of a protein-free solution containing all the substances freely dissolved in the plasma in the same concentration as in the plasma with the exception of the small difference due to the Donnan effect.

b) active reabsorption in the tubules of the larger part of this filtrate, i.e. water and other substances necessary for metabolism or for the maintenance of the electrolyte balance in the body.

c) passive rediffusion of certain substances through the tubules, this diffusion being entirely dependent on the degree of concentration of the respective substance in the tubular urine and on its diffusibility.

d) active secretion through the tubular cells of other substances, either normally present in the plasma or artificially administered.

e) a synthesis of certain substances in the kidney.

### Glomerular Filtration

Ludwig's and Cushny's hypothesis of glomerular filtration was first confirmed by the investigations of Wearn and Richards (1924). They were able to demonstrate in punctates from the glomeruli of the frog that protein is lacking but that chloride and glucose are present in approximately the same concentrations as in the plasma. Using the same method, it was later shown that the concentrations of urea (Walker and Elsom 1930) inorganic phosphate and reducing substances (Walker 1933, Walker and Reisinger 1933) are identical in the plasma and in the content of the glomerular capsules of the frog. Bordley and Richards (1933) and Bordley, Hendrix and Richards (1933) demonstrated the presence of creatinine and uric acid in the fluid of the glomerular capsule of the frog. The electrical conductivity in the glomerular punctate, in the

blood plasma and in the artificial ultrafiltrate from the blood plasma of the frog and *Necturus maculosus* were also demonstrated to be identical (Bayliss and Walker 1930) as well as the total molecular concentration (Walker 1930, White 1932). Ekehorn (1931) was of the opinion that the »protein proof» described by him was an incontrovertible proof of the actual occurrence of ultrafiltration. In injuries to the glomerular membrane the protein is filtered by an entirely passive process. This process is, for example, extremely strong in the glomerular capsule in definitely dead kidneys and usually increases with the degree of artificial injury to the glomerulus. A purely passive filtration of protein in the glomerulus nevertheless entirely excludes all secretion of water and salts in it. Ekehorn (1938) writes: »If two fluids, as is the case in the glomeruli, are only separated by a membrane less than  $1\mu$  thick, and if this membrane even allows the passage of extremely large protein molecules, there cannot possibly exist any secretory difference between the two fluids. Should the membrane cells nevertheless endeavour to secrete more or less fluid than corresponds to an ultrafiltration, or to secrete a fluid of another composition than that of an ultrafiltrate with regard to the diffusible substances, this difference must necessarily be evened out already *in statu nascendi* owing to diffusion and osmosis through this extremely permeable membrane. Thus, both as regards quantity and composition, the protein-containing glomerular fluid has the nature of a filtration product from the blood.»

Further support for the occurrence of filtration in the glomeruli is given by Marshall and Grafflin (1928). In their investigations of the urine of aglomerular fish they were able to ascertain that these were incapable of excreting ferrocyanide or glucose, the latter not even after intoxication with phlorhizin.

All the forementioned investigations were carried out on amphibia. The question then arises: is there no corresponding direct evidence that the same filtration process occurs in mammals and in man? Direct evidence is rare. Starling



and Verney's experiments (1925) with artificially perfused kidneys point towards ultrafiltration but are scarcely conclusive. Bayliss, Kerridge and Russell (1933) demonstrated that the glomerular membrane in the cat, the rabbit and the dog is permeable for molecules with a molecular weight below 70,000, thus what could be expected of a semi-permeable membrane. Other evidence is of a more indirect nature, consisting partly of conclusions drawn from comparative anatomical investigations of the structure of the glomerulus in various species and partly from results of so-called «clearance» determinations.

The term «clearance» was introduced by Møller, McIntosh and Van Slyke (1928) as a measure of the ability of the kidney to excrete urea. Clearance was defined by them as «the volume of blood which one minute's excretion of urine suffices to clear of urea». The interpretation of clearance has later been widened to include the expression of the excretion of various substances and can thus, according to Smith (1937) be defined as «the minimum volume of blood required to furnish the quantity of substance excreted in the urine in one minute's time».

Several factors influence the effectivity of the kidneys. Clearance may be said to be a method of expressing the effect with regard to the concentration in the plasma. Instead of stating how much is excreted per minute in grammes, we state how much plasma this quantity corresponds to in regard to the concentration in the plasma of the substance. Obviously, a certain quantity of plasma is not absolutely cleared of the substance. Such a clearance is only assumed for the sake of convenience.

If a substance existed that was freely soluble in plasma and entirely filtrable and which, during further passage through the tubules was not reabsorbed or any of the substance secreted through them, that quantity of the substance that was excreted in the bladder urine in one minute would be equal to the quantity filtered in the glomeruli during the same time and its clearance would be equal to the volume of the filtrate.

These clearance investigations have had considerable importance in determining whether filtration actually takes place in higher animals and in man. Several workers (Richards, Westfall and Boll 1935, 1936, Shannon 1935, Van Slyke, Hiller and Miller 1935) have demonstrated that the clearances for inulin, creatinine and ferrocyanide are identical in the dog, inulin and creatinine identical in the rabbit (Kaplan and Smith 1935) and sheep (Shannon 1938) and in anthropoid apes following intoxication with phlorhizin (Smith and Clarke 1938) and inulin, sorbitol and mannitol identical in man (Smith, Finkelstein and Smith 1940). After phlorhizin intoxication, glucose, xylose and creatinine show equal clearances in man (Shannon and Smith 1935) and the figures are approximately of the same order of magnitude as for inulin, sorbitol and mannitol.

The probability that these substances are handled uniformly by the kidney is naturally far greater than that they are excreted by separate mechanisms and that the clearance figure is nevertheless the same. According to our present knowledge, inulin fulfills all the criteria of an ideal test-substance suitable for filtration determinations (v. p. 31) and it has therefore been possible to determine the rate of glomerular filtration. Figures varying between 120 and 145 ml per minute are stated as normal for persons with healthy kidneys (Smith 1937, Josephson and Lindahl 1943, Hogeman 1943). Moreover, these figures are in good agreement with those that can be expected from comparative anatomical investigations and calculations of the amount of filtrate per gramme of kidney weight which has been found to be approximately similar in all animal series. Strangely enough, these calculated filtration rates lie almost exactly at the level calculated by Heidenhain (1883) as necessary if the filtration theory were correct, figures that he considered to be a more incontrovertible proof than any other of the absurdity of the whole theory!

The possibility of such a large filtration per minute was demonstrated by Rehberg (1926) amongst others. On the

basis of Vimtrup's calculations, published later (1928), of the number of glomeruli in the kidneys in man (2,000,000) and of the filtrating surface ( $1.56 \text{ m}^2$ ) he came to the conclusion that a filtrate of the order of magnitude of 120-125 ml per minute is fully credible. Similar calculations were also made by Ekehorn (1938) who came to approximately the same result. According to Smith (1937) this corresponds to about one drop per second through a filter surface of  $20 \times 20 \text{ cm}$ .

Are such forces available that can perform an ultrafiltration? Ludwig, as early as 1844, considered the blood pressure to be a sufficient source of energy and this has been confirmed in later investigations by Starling and Verney (1925), Hayman (1927) and Winton (1931b) amongst others. The pressure in the glomerular capillaries is approximately two-thirds of the arterial pressure and this is counteracted by the colloid-osmotic pressure, 25-30 mm and the intracapsular pressure, approximately 5-10 mm (Best and Taylor 1937). The effective filtration pressure in man would thus be approximately 30-40 mm Hg at normal blood pressure. By decreasing the blood pressure or by increasing the urethral pressure it has been possible to affect experimentally the volume of filtrate (Dreyer and Verney 1923, Winton 1931 a).

Direct examination of the glomeruli of the frog has shown that only a certain number function simultaneously (Richards and Schmidt 1924, Ekehorn 1931, Okkels 1930). It is obviously impossible to perform similar direct investigations in mammals and in man, but there is reason to suppose that the conditions differ and that the volume of the filtrate is kept fairly constant. Small variations in the blood pressure and the colloid-osmotic pressure are regulated by means of tonus changes in the efferent arterioles which in this way endeavour to assure a constant filtration pressure (Chasis, Ranges, Goldring and Smith 1938, Schroeder and Steele 1939). Greater changes in the blood pressure cannot, however, be entirely compensated by this mechanism, which nevertheless carries out a compensation as

far as is possible by an increase in the so-called filtration fraction (F.F.), i.e. the percentage of plasma that is filtered in the glomeruli (Smith 1943, Moberger 1945). Variations in the blood flow of the kidney are not always manifested in changes in filtration rate owing to the fact that tonus changes occur in the afferent or efferent arterioles. A decreased blood flow can, for example, be associated with an increased tonus in the efferent arterioles, resulting in a higher filtration pressure, an increased filtration fraction and an almost constant filtration rate (Smith 1943).

### Tubular Reabsorption and Diffusion

In its passage through the tubules the glomerular urine is concentrated to 98-99 per cent. This process was considered by Ludwig (1844) to be a mere diffusion, but later workers have shown that the process is more complicated. Wearn (1922) demonstrated that a redning substance occurred in the glomerular urine of the frog following injection of glucose but that the bladder urine remained free from it. Mayrs (1923) found in rabbits, whose tubules had been rendered incapable of function by means of phlorhizin, that glucose appeared in the urine in approximately the same concentration as sulphate — which he considered to be freely filtrable but not subject to tubular reabsorption — but in a higher concentration than urea. He concluded that glucose could no longer be reabsorbed, whereas some urea could be. Rehberg (1926) investigated the excretion of urea and chloride and came to the conclusion that urea diffuses to some extent through the tubules whereas chloride is actively resorbed in the proximal tubules together with some water, the remainder being reabsorbed more distally. Marshall and Grafflin (1932) demonstrated the same process in glomerular fish. On puncture of the tubules in frogs and *Necturus*, Walker, Hindson, Findley and Richards (1937) found, on the contrary, that the chlorides are reabsorbed in the distal tubule, whereas the glucose is reabsorbed in the proximal segment. Ni and Rehberg (1930) also

studied this question and concluded that this process is complete at a glucose-plasma concentration below 200 mg per cent and shows a lower percentage rate at rising concentrations. In their opinion the maximum of reabsorption is governed by a »limiting factor» conditioned by the difference in concentration between the blood and the tubular urine. Lundsgaard (1933 a, 1933 b) believed that the reabsorption of glucose depends on a phosphorylation process in the tubules, analogous to that postulated by Verzář and his school with regard to the reabsorption of carbohydrates through the intestinal wall (Verzář 1931, Wilbrandt and Laszt 1933). According to Lundsgaard this phosphorylation process is inhibited by phlorhizin and since this substance is more concentrated in the kidney than in any other organ, its effect is strongest there. MacKay and MacKay (1936) were of the opinion that a threshold for the absorption of sodium chloride exists in the rabbit which is not, however, fixed but varies according to the flow of urine. Shannon and Fisher (1938) reinvestigated the question of the mechanism of glucose reabsorption. They were able to demonstrate that this is limited in the dog to a certain quantity per time unit and that if more glucose is offered to the tubules the excess is excreted in the urine. It was also possible to show, by experiments with high plasma concentrations of glucose lasting for several hours, that this process is not subject to »fatigue» owing to continued hyperglycaemia. By experimentally lowering the filtration volume to 50 per cent and nevertheless obtaining a constant reabsorption figure, the forementioned writers considered that they had proved that the diffusion gradient between blood and urine postulated by Ni and Rehberg (1930) could not cause the limiting of glucose reabsorption. They put forward another theory, i.e. that glucose undergoes a reversible combination with some element in the tubular cell which is found there in a constant but limited amount. The subsequent decomposition of this complex or compound limits the rate of glucose transfer from tubular urine to blood. The process can be expressed in the formula  $A + B \rightleftharpoons AB \rightarrow T_r + B$ , where A is

the glucose in the tubular urine, B the cell element, AB the complex formed and  $T_r$  the glucose distal of the initial reaction. This theory has proved to be applicable not only to glucose but to all substances having a maximal tubular reabsorption or secretion.

Ascorbic acid is reabsorbed in the same way as glucose (Ralli, Friedman and Rubin 1938, Friedman, Sherry and Ralli 1940, Ahlborg 1946) although probably not by the same mechanism. Selkurt (1944) demonstrated that on maximal reabsorption of glucose, ascorbic acid reabsorption is initially totally blocked, as is also the case with simultaneous maximal excretion of *p*-amino hippuric acid, and then gradually rises. Although their respective tubular mechanisms do not mutually interfere, a simultaneous maximal reabsorption or excretion of glucose and *p*-amino hippuric acid lowers the reabsorption of ascorbic acid to a greater extent than either substance separately. It must therefore be concluded that the interference observed does not depend on competition for a specific transport mechanism. The cause is instead presumed to lie in the system that delivers energy for the transport mechanisms of the tubules.

Pitts (1943) made a closer study of the renal reabsorption processes for amino acids. He found that the amino acids glycine, alanine, glutamic acid and arginine are in all probability reabsorbed by a common mechanism although with considerable variations in the rate of absorption. He considered this to depend on the varying speed with which the different amino acids are bound to some cell component common to their reabsorption.

Maximal tubular reabsorption is nowadays generally designated in the literature by  $T_m$  (Smith, Goldring and Chasis, 1938). This term also partly includes what Cushny (1917) defined as "threshold substances" a term now used in various connexions. According to Barclay, Cook and Kenney (1947) " $T_m$  is most suitably used to describe the limiting rate of reabsorption (or secretion) of a substance when two components only are involved

(either filtration and reabsorption or filtration and secretion). The term »threshold plasma level« is used when three components are involved (filtration and secretion-reabsorption or diffusion) and the quantity of reabsorbed substance should then be stated per 100 ml of the glomerular filtrate.

Our knowledge of the tubular reabsorption of water is as yet incomplete despite extensive study of the problem. Peter (1909) was of the opinion that the thin segment of Henle's loop is the site of this process. This is in agreement with Crane's (1927) statement that this thin segment is only present in species capable of producing hypertonic urine. Walker and Hudson (1937) found, however, in their puncture experiments on the frog that glucose is partly concentrated in the proximal tubule and interpreted this as a water reabsorption at this site. Water reabsorption in the distal segment of the tubule is also probable (White 1929). Nowadays it is generally assumed that part of the water is reabsorbed in the proximal tubules and part in the thin segment regulated by the antidiuretic hormone formed in the posterior pituitary lobe. This opinion is supported by the observations of Heller (1940) who studied the distribution of the antidiuretic hormone in vertebrates and concluded that a certain correlation could be traced between the development of Henle's loop and the quantity of antidiuretic hormone in the posterior pituitary lobe.

### Tubular Secretion

Marshall and Vickers (1923) and Marshall and Crane (1924) considered that they had demonstrated that phenol red is excreted in amphibia and mammals by tubular secretion and that this secretion is less when the plasma concentration is high than when it is low. They interpreted this as a saturation of the secreting cells in the kidney with the substance in question. Already in 1922 Haan showed that the concentration of phenol red in the urine following injection was so high that a glomerular filtrate of 56 litres per hour must be postulated to explain this output. Moreover,

since phenol red is bound to the serum proteins, the results of the investigation were taken as a proof that the filtration-reabsorption theory must be incorrect. Marshall and Grafflin (1932) demonstrated the secretion of magnesium, sulphate, creatinine and phenol red in aglomerular fish. The last-mentioned substance also showed an upper limit indicating some limitation in the excretion mechanism (Bieter 1933). Chambers and Kempton (1933) showed in *in vitro* cultures of chick tubules that phenol red is secreted in the interior of the tubular lumen if it is added to the culture medium but that it is not absorbed into the cells if it is injected into the lumen. Using the clearance method, Shannon (1935) demonstrated that in the dog phenol red is excreted to a great extent by the tubules. At plasma concentrations of phenol red between 0.5 and 1.5 mg per cent. approximately 83 per cent was excreted by secretion, whereas this part fell to 35 per cent if the plasma concentration was increased to 40 mg per cent. He also found an upper limit for this secretory capacity similar to that for the tubular reabsorption of, for example, glucose. Contrary to the latter, this was not affected by phlorhizin. Goldring, Clarke and Smith (1936) made similar investigations in man and found the same results, i.e. at low plasma concentrations of the substance its clearance was 3.2 times greater than the inulin clearance, whereas in rising concentrations a self-depression of the clearance occurred, both absolute and in relation to inulin. Determinations of this maximal secretion in man — tubular excretory mass ( $T_m$ ) — were later performed by Smith, Goldring and Chasis (1938).

In 1934 Elsom, Bott and Landis published the results of their investigations on the excretion of organic iodine compounds in the dog and in man. It was then shown that two of the compounds investigated, diodrast and hippuran, were excreted in principle in the same manner and that their respective clearance figures were many times higher than the creatinine clearance which — at any rate in the dog — is identical with the glomerular filtration. This fact indicated a considerable tubular secretion of these iodine com-



pounds and the secretory process showed the same characteristics as those of phenol red, i.e. constant »tubular excretory mass» at high plasma concentrations, high clearance at low plasma concentrations and »self depression» at rising concentrations (Smith, Goldring and Chasis 1938). In a later publication (1936) Elsom, Bott and Shiels confirmed their observations in the dog on a larger material. They demonstrated at the same time that the secretory process is not bound to the iodine molecule since the clearance for sodium iodide is considerably lower than that of creatinine. It has later been shown that other substances than organic iodine compounds are excreted extensively by tubular secretion (Smith et al. 1945).

A common characteristic of all substances that undergo tubular secretion is that their clearance at low plasma concentrations is higher than the inulin clearance and that at rising concentrations the clearance falls and approaches inulin clearance. The clearance of substances that are reabsorbed or diffused through the tubules is, on the contrary, lower than the inulin clearance. It is a controversial question whether tubular secretion occurs in physiological conditions, i.e. if some substance normally present in the plasma is excreted by this mechanism. The majority of American workers (Shannon, Smith, and others) consider that creatinine is partly secreted by the tubules. This is energetically denied by others, especially by Ekehorn (1944). This question is more extensively treated later on.

### Synthesis of Certain Substances in the Kidneys

Bunge and Schmiedeberg (1876) were the first to demonstrate by perfusing the kidneys of dogs that hippuric acid is formed under certain experimental conditions, and that the kidney is capable of performing this synthesis. In the synthesis the reactants were shown to be benzoic acid and glycine. If the benzoic acid enters into higher homologues, the kidney can also carry out the necessary  $\beta$ -oxidation before the synthesis (Snapper and Greenbaum, quoted

from Berglund and Medes 1935). The first-mentioned writers could also prove on perfused human kidneys that the synthesis of hippuric acid takes place at this site.

Ammonia can also be formed in the kidneys, as was shown by Nash and Benedict (1921). The mother substance for this synthesis is unanimously considered to be urea (Krebs 1936). As Van Slyke et al. (1935d) and Pitts (1936) amongst others pointed out, this fact can be of some significance in calculating the clearance of urea. (v. Urea Clearance). Moreover, this ammonia synthesis is of tremendous importance, particularly in acidosis. The whole question of the rôle of the kidney in regulating the pH of the urine and the electrolyte balance of the body is extremely complicated and falls outside the scope of the questions to be discussed in the present paper.

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## Renal Functional Tests

In the course of years a number of methods have been used to investigate the functional condition of the kidney. In the beginning tests were made simply to determine the concentrations in the blood of such substances whose concentration depends on renal function. The kidney, like other organs, has a considerable reserve of efficiency. The blood concentration can therefore be normal although, for instance, one of the kidneys has been removed. Therefore, it was later found more satisfactory to expose the kidneys to certain tolerance tests. Still later it was found advantageous to control such tolerance tests by taking blood samples simultaneously. On the basis of these approaches to the problem, renal functional tests can be divided into three main groups:

- A. Tests by examination of the blood or urine.
- B. Tolerance tests.
- C. Tests by means of simultaneous investigation of the blood and the urine («clearance tests»).

## Functional Tests by Examination of the Blood or Urine

The most important of these tests is indubitably the determination of non protein nitrogen or of blood urea. As long ago as 1823, Prevost and Dumas pointed out that the blood urea rises after bilateral nephrectomy in animals, and Bright et al. (1836) noticed a rise in certain types of nephritis. The normal concentration of urea nitrogen in the blood varies according to different writers. McLean (1915) gives 14-23 mg per cent as the normal value, and Myers, Fine and Lough (1916) 15 mg per cent as the upper limit, an opinion shared by Mosenthal and Lewis (1916), Trumper and Cantarow (1932) and Ohlson (1940). Schwarz and McGill (1916) give somewhat higher values, i.e. 12 mg per cent as an average and only a value in excess of 25 mg per cent as definitely pathological. Volhard and Becher (1929) give 10-20 mg per cent as a normal value, as do Cameron and Gilmour (1935). MacKay and MacKay (1927) found in their investigations that the value was somewhat lower for women than for men, i.e. 12 and 16 mg per cent respectively and that a tendency to higher figures occurred with increasing age. The latter fact is also pointed out by Lewis and Alving (1938) who found an increasing rise in men over the age of 40, i.e. from 12 mg per cent at 40 years of age to 17.6 mg per cent at the age of 89.

Non protein nitrogen is used in many clinical investigations, particularly in Germany and Scandinavia, instead of urea nitrogen. The normal values are also somewhat variable, possibly partly depending on the method of determination used. Foster (1913) thus gives 32.5 mg per cent as an average value and 44 mg per cent as the upper limit, whereas Folin and Denis (1913) and Tileston and Comfort (1914) give the latter as 30 mg per cent. Mosenthal and Lewis (1916) state 35 mg per cent to be the upper limit and Volhard and Becher (1929) 40 mg per cent.

In consideration of the fact that the urea nitrogen is

normally approximately 50 per cent of the non protein nitrogen and in retention rises more than the latter (v. Volhard and Becher 1929), Mosenthal and Bruger (1934) worked out their »urea ratio», i.e. the urea nitrogen as a percentage of the non protein nitrogen. They considered this »ratio» to be a more sensitive measure of incipient renal insufficiency than single determinations of non protein nitrogen or urea nitrogen. These simple blood analyses are not, however, a sensitive measure of renal function, as is demonstrated by the fact that fairly normal figures are obtained even in cases where one kidney is lacking if the other is healthy. According to Hollen and Rehberg (1929) the concentration of urea nitrogen in the plasma starts to rise when the glomerular filtrate falls below 100 ml/min and the urea shows very high values when it falls below 60 ml/min. They emphasize, however, that a normal urea nitrogen value does not exclude serious impairment of renal function. MacKay and MacKay (1927) are, however, of the opinion that the blood urea does not begin to rise until the renal parenchyma is reduced to 50 per cent, an opinion shared by Møller et al. (1929b) and Van Slyke et al. (1930a).

The concentration of other substances in the blood has been used as a renal functional test. Thus the *creatinine retention* is considered by some workers to have a greater prognostic value than non protein nitrogen or urea nitrogen (Volhard and Becher 1929) although a parallelism between the different figures even by this test is not always obtained (Patch and Rabinowitch 1928). According to some statements, the concentration of *uric acid* in the blood increases earlier than that of any other substance in incipient renal insufficiency. This fact is, however, of less importance since a number of other pathological conditions can also result in increased concentration (Volhard and Becher 1929). Johnston (1931) who investigated 30 cases of renal disorders concluded that a normal concentration of uric acid can occur even if 80 per cent of the renal function is lost and that, when an increase in the concen-

tration of uric acid occurs, there is no relation between the degree of this increase and the decrease of renal efficiency. Wakefield (1929, 1931) is of the opinion that in incipient renal insufficiency *inorganic sulphate* increases in the serum earlier than urea or creatinine and he found good correlation between the increase of inorganic sulphate and decrease of renal function determined by urea clearance and phenol-sulphonphthalein excretion. Determination of *amino nitrogen* is, however, of very little importance (Kirk 1933).

It is as a rule impossible to obtain any true picture of renal function by means of chemical or physical investigations of the urine. An exception is, nevertheless, the determination of the maximal concentration capacity of the kidneys, which will be discussed in connexion with the water test (v. p. 17). Important data can, however, be obtained through microscopical examination of the urinary sediment. A refinement of the sediment investigation method was introduced by Addis (1925). This consists of a count of the cellular elements and casts in a counting chamber. The determination is usually made on 12-hour urine (night urine) but neither exercise nor a high-protein diet appears to affect the sediment (Addis 1926). The excretion of cells and casts is, however, according to MacKay (1936) more constant during the night than during the day. He believed this to depend on the existence of a certain 24-hours' rhythm that gives varying results during this period. Normal values are given by Addis (1925), Goldring and Wyckoff (1930), Lytle (1933) and Næraa (1931) amongst others.

To sum up it may be said of this group of functional tests that the determination of non protein nitrogen or of urea nitrogen gives rapid information as to whether a severe functional disturbance is present or not, and that quantitative sediment determinations according to Addis' method are valuable in order to follow the course of certain renal disorders. The normal values given as follows are taken from the literature and must naturally only be regarded as approximative. Moreover, it is evident from the foregoing that different values are given by different writers.

### Normal Values According to the Literature

*Urea nitrogen*: 8-15 mg per cent;  
 upper limit: 20 mg per cent.  
*Non protein nitrogen*: 20-35 mg per cent;  
 upper limit: 40 mg per cent.  
*Quantitative sediment*: Red corpuscles: 65,000-170,000;  
 upper limit: 1 million/mm<sup>3</sup>.  
 White corpuscles and epithelial cells: 320,000-650,000;  
 upper limit: 1 million/mm<sup>3</sup>  
 Casts: 650-2,000; upper limit: 9,000/mm<sup>3</sup>.

### Tolerance Tests

The two most widely used tests in this group are the water or concentration test and the phenolsulphonphthalein test.

The *water test* was introduced clinically by Volhard (1918). The subject is given 1.5 litres of water to drink over a period of 30-45 minutes. The urine is then collected every 30 minutes for the first four hours and thereafter every two hours for 12 or 24 hours. The volume and specific gravity of each specimen are measured. No further fluid is then given during the test period and it is therefore possible in this way to obtain a combined dilution and concentration test. Volhard's criteria for the normal kidney are: excretion of all the water during the first four hours, dilution of the urine to a specific gravity of 1.002-1.004 and then concentration to 1.026-1.030. The main sources of error in this test are manifest oedema or incipient oedema, high external temperature which promotes sweating or considerable evaporation, etc. There are many modifications of Volhard's original test (Siebeck 1919, Lundsgaard 1920, Munk 1925, Strauss 1927, Rosenberg 1927) but the variations are usually minor ones. According to Pratt (1926) Volhard's criteria are too strict since, according to the former, dilutions of only 1.005-1.008 have occurred in patients despite healthy kidneys. Pratt explains this by assuming an unusually large quantity of solid substances being excreted during diuresis. On account of the varying

results of the dilution test, many workers have discarded the water tolerance test and have instead studied the kidney's ability to produce strongly concentrated urine which, moreover, according to Volhard, is the best measure of its activity. It should, nevertheless, be emphasized that there is naturally nothing unsuitable in performing the dilution test as well, and that it gives certain information. Mosenthal (1915) used a test-meal and collected the day urine in 2-hour portions and the night urine in one portion. His criteria for a normal test were a specific gravity of 1.018 or more, a variation of .009 or more between the lowest and highest values for specific gravity, a fairly small volume of night urine — 400 ml or less — with a high specific gravity, i.e. 1.018 or higher. Addis and Shevky (1922) determined the specific gravity of the urine after 24 hours' water deprivation and found that it was higher than 1.026 in 100 per cent and higher than 1.028 in 95 per cent. Lashmet and Newburgh (1932) also made determinations of the night urine after three days' preparatory stay in bed on a specific diet. They required a specific gravity of 1.029-1.032, and an increase of the non protein nitrogen was only registered when this dropped to 1.010. Alving and Van Slyke (1934) made a critical study of the concentration and dilution tests and came to the following conclusions: all concentration tests are sensitive, Mosenthal's being possibly the least sensitive. If the specific gravity reaches 1.026, renal function is as a rule normal. There is no necessity to perform the dilution test, which is less sensitive and affords no additional information to that given by the concentration test. Freyberg (1935) also recommends the concentration test as the best and most sensitive measure of renal function.

The *phenolsulphonphthalein* test was first described by Rowntree and Geraghty (1910). When this dye is injected intramuscularly, 60 per cent or more is normally recovered in the urine within the succeeding two hours. According to the forementioned writers: »when the drug was continuously excreted in traces or not at all, a grave prognosis was to be given even without signs of uremia».

There is plentiful literature in existence regarding the value of this test, particularly after Folin (1913) elaborated a more satisfactory technique. American workers in particular consider the test to be extremely sensitive (Rowntree and Fitz 1913, Chace and Myers 1916) but it also has its warm adherents in Scandinavia (Lundsgaard and Møller 1925). Mosenthal and Lewis (1916) and Van Slyke et al. (1930) are of the opinion that this test does not give more enlightenment than other functional tests and that its sensitivity is less than that of the concentration or urea clearance test. Volhard and Becher (1929) consider that it is not «absolutely necessary» for the determination of renal function and approximately the same opinion is held by Freyberg (1935) who nevertheless states that the excretion determined during the first 15 minutes after the injection gives more accurate figures than the determination after two hours. He gives the normal figure after 15 minutes as 28-29 per cent.

Interest in the phenolsulphophthalein test has nevertheless increased during the last ten to twelve years since this substance, phenol red, is also used in clearance determinations and has contributed to the solution of certain problems regarding the physiology of the kidney (v. p. 10).

Nyiri's *thiosulphate test* (1923) is perhaps that of the other tolerance tests which has been most used and debated (Iversen 1925, Warburg 1925, Holbøll 1925). Since this, as well as a number of other tolerance tests, is very little used nowadays reference is made to Volhard and Becher (1929) for a closer study.

To sum up, it can be said regarding these tests that the water test — or more correctly the concentration test — can give valuable information regarding the functional condition of the kidney but that it does not permit any differential diagnosis of various renal disorders since only one function is registered, i.e. the ability of the kidney to concentrate water and salts. Another disadvantage is that a lower limit exists for this test in cases of isosthenuria and no further deterioration of the renal function can therefore be discovered. Of the other



tolerance tests, the phenolsulphonphthalein test gives the most rapid enlightenment regarding the function of the kidney, particularly in Freyberg's modification with determination of the excreted amount after the first 15 minutes (normal figure: 28-29 per cent).

## Functional Tests by Means of Simultaneous Investigation of the Blood and the Urine ("Clearance Tests")

### Urea Clearance

Ambard (1910) attempted by means of his well-known formula to express numerically the relation between the urea concentration in the blood and its rate of excretion in the urine. The pith of Ambard's rule can be expressed as follows: the ratio between the concentration of urea in the blood and the square root of the 24-hour excretion of urea is constant and equals 0.06--0.09 in persons with normal kidneys. The fact that the quotient is proportional to the square root of the excretion of urine is based on purely empirical grounds. It is naturally possible that this may be found, on further investigation, to be governed by some other function. Nevertheless, in renal impairment there is an increase in this figure as an indication of the inability of the kidney to eliminate urea in proportion to its concentration in the blood.

Ambard's hypothesis was not confirmed by Marshall and Davis (1914) who found that in normal animals the rate of urea excretion is proportional to the concentration in the blood only when a sufficiently large flow of urine is maintained. Addis and Watanabe (1916) were of the opinion that the ratio 
$$\frac{\text{Urea excretion, g per one hour}}{\text{Urea g in 100 ml of blood}}$$
 is a measure

of the activity of the kidney provided that the blood flow through the kidneys is constant. They had three conditions: 1) since the ratio varies with such controllable factors as diet, intake of fluid, etc., these should be standardized; 2) since the ratio also varies with other and uncontrollable factors, it is necessary to use the mean figure of several determinations; 3) since the method is used to ascertain pathological changes in the kidneys of various individuals, it must be per-

formed under conditions that emphasize such differences to as great an extent as possible. Therefore urea loading should be used. They were able to demonstrate by their determinations that in the rabbit a lower figure after unilateral nephrectomy was only obtained following urea load; without such a load there was no difference in the figure obtained before and after the operation. Austin, Stillman and Van Slyke (1921) and Møller, McIntosh and Van Slyke (1928) developed Addis' ratio further and demonstrated that this ratio only varies in direct proportion to the volume of urine when this latter exceeds 2 ml per minute (=augmentation limit). When it falls below this figure the urea excretion is directly proportional to the square root of the urine volume. They set up two formulae for the calculation of this excretion, which was then called "urea clearance".

$$1) \text{ "Maximum clearance": } \frac{\text{Urea \% in the urine} \times \text{urine volume/min}}{\text{Urea \% in the blood}}$$

can be used when the urine volume exceeds 2 ml per minute. This is essentially the same as Addis' ratio except that here the excretion is calculated per minute instead of per hour. Actually, the formula implies that the concentration of urea in the urine during one minute is divided by the concentration of urea in the blood per one ml. It is the ratio between these quantities that is determined.

$$2) \text{ "Standard clearance": } \frac{\text{Urea \% in the urine} \times \sqrt{\text{urine volume/min}}}{\text{Urea \% in the blood}}$$

to be used when the urine volume is less than 2 ml per minute. By using the square root of the volume of urine, one obtains an expression of the urea clearance which approximates a standard volume of one ml per minute. This is approximately the normal urinary volume in a healthy individual under ordinary conditions. A functional change must obviously occur gradually so that instead of being proportional to the volume of urine it is proportional to its square root when the volume decreases. It is not possible to conceive of any sharply defined borderline. Under such conditions

the formulae can only give approximate results particularly in the neighbourhood of a volume of 2 ml.

The accepted average for the »maximum» clearance is 75 ml per minute (50-104 ml) and 54 ml per minute (25-69 ml) for the »standard» clearance. Instead of stating the excretion in ml per minute, it is nowadays usual to give this as a percentage of the normal figure, it thus being easier to compare the figures for varying volumes of urine. By introducing a correction to a standard body size of 1.73 m<sup>2</sup>, more easily comparable figures are obtained (McIntosh, Møller and Van Slyke 1929). MacKay (1932) thus pointed out that renal function is directly proportional to the surface of the body, to which the weight of the kidney is in direct proportion, a fact that has been established by special investigations.

In young premature and full-term infants the urea clearance is lower than in older children (Gordon 1942). Nor is it constant throughout the 24-hour period. According to MacKay (1929) it is maximal and the variations minimal between 9 a.m. and 12 noon and the investigation should therefore take place during this period. On the other hand, Bruger and Mosenthal (1932a) point out that urea clearance varies considerably in healthy individuals from hour to hour and from day to day. The most important fact is that the figures can vary since it has been demonstrated that these variations decrease with increased renal impairment, as is the case for the specific gravity. It is thus hardly possible to give the figure as a percentage of the normal clearance since the latter is not found in one figure but in a latitude.

Diet is of considerable importance for urea clearance. Addis and Drury (1923) found that the maximal urea clearance can be increased by a high-protein diet and this was also demonstrated by Jolliffe and Smith (1931) and Shannon, Jolliffe and Smith (1932) in the dog. Van Slyke, Rhoads, Hiller and Alving (1935c) found that in the dog the urea clearance increased 2-3 times after a high-protein diet and that this was parallel to an increase

in the renal blood flow. Since in their opinion urea clearance is chiefly dependent on the renal blood flow, they believe that the clearance increase is possibly part of the general phenomenon of increased circulation following a high-protein diet.

Cope (1933 a) demonstrated that in normal individuals the urea clearance does not appreciably increase with a moderate increase of protein in the diet but falls if the protein intake is decreased from 75 g to 40 g per day and that this decrease is absent in nephritic patients. Goldring, Razinsky, Greenblatt and Cohen (1934) made the same observation in healthy individuals. Van Slyke, Page, Hiller and Kirk (1935) also found low urea clearance with a low-protein diet if the clearance was calculated on the excretion of urea alone. Since, however, it is assumed that ammonia is formed in the kidney by urea, it is according to these writers more correct to calculate the excretion of urea plus ammonia. From a technical standpoint this is also easier and there is then no decrease in the clearance on a low-protein diet. Farr (1936) observed an increase in the urea clearance in nephrotic children on a high-protein diet, but no increase if only urea was given. The increase seems therefore to be conditioned by other metabolites than urea.

Dominguez (1935) considered that an increase of urea clearance following a high-protein diet is essential for maintaining the nitrogen equilibrium of the body. If there was no increased clearance, the urea would inevitably be retained unless the flow of urine increased in proportion to the concentration of urea, an opinion which seems to be the object of considerable doubt. Simultaneous ingestion of large quantities of dextrose does not affect the clearance (Bruger, Mosenthal 1932 b). It varies considerably from hour to hour in normal individuals as the same writers pointed out earlier (Bruger, Mosenthal 1932 a). A restriction of salt, however, causes a decrease and the administration of sodium chloride an increase of the urea clearance as well as a decrease of urea nitrogen in the blood (Landis, Elsom, Bott and Shiels 1935). The explanation given

by Ekehorn (1945) is that in hypochloraemia more urea rediffuses in the proximal tubule since the concentration of sodium chloride in the tubular urine is lower than normal. After increasing the concentration of sodium chloride, urea rediffuses to a smaller extent and a higher clearance is obtained. This seems to be a reasonable explanation.

The usefulness of »maximum» and »standard» clearance is discussed by several writers. Summerville, Hanzal and Goldblatt (1932) determined the »augmentation» limit in the dog and found it to be 0.45 ml per minute. By using this figure as a standard volume of reference in all volumes of urine, both formulae can be avoided and it becomes possible to include all figures under a common term, i.e. urea clearance. Chesley (1937) points out that the square root formula for standard clearance gives erroneously low results if the urine flow falls below a certain critical volume, which for adults is about 0.35 ml per minute. The ratio: Urea % in the urine/Urea % in the blood, is then reversed and decreases with a further decrease of the volume. He therefore recommends that in such cases the test should be discarded. These formulae are also criticized by Bing (1946) and Williams (1946) but their objections are not sustained by Van Slyke (1947) who considers that his original formulae are more accurate than those of Bing and Williams. As emphasized in the foregoing, a valid formula must include several terms, of which one is proportional to the volume of urine and one to its square root. The first term must play the principal rôle in high figures and the second in low figures. It is difficult to envisage the situation with extremely low figures.

What is the use of urea clearance? Cushny was first (1917) of the opinion that the urea is filtered in the glomeruli but not reabsorbed in the tubules, i.e. a typical »non-threshold» substance. In a later edition of his monograph (1924) he has, however, changed his opinion. Mayrs (1922) established that less urea than sulphate, phosphate and creatinine is concentrated in the urine by the kidney and that by raising the urethral pressure the urea concentration is further re-

duced. It has been established through the investigations of Rehberg (1926), Hollen and Rehberg (1931), Shannon (1936), and others, that a considerable amount of the urea is rediffused or reabsorbed by the tubules, about 40 per cent with a large volume of urine and still more with a low volume. Shannon (1938) made a further study of this condition in the dog. He came to the conclusion that a mere diffusion process cannot explain the entire loss of urea but he supposes therefore that a minimum of two independent processes might operate. One would be bound to the reabsorption of water that takes place in the proximal tubule, the «fixed obligatory» water reabsorption that is responsible for the deficit of urea excretion found when the volume of urine is large. The other would be a diffusion in the distal tubule connected with the «facultative» reabsorption of varying quantities of water taking place in that area. This latter process would then be responsible for the further decrease of the urea output in the urine found with a low flow of urine. Dole (1943) reached the same conclusion regarding the excretion of urea in the dog and in man.

Urea clearance is thus no direct measure of glomerular filtration but of this filtration and the permeability of the tubular wall. This fact does not, however, prevent the test having been used with considerable success as a renal functional test. Van Slyke, McIntosh, Møller, Hannon and Johnston (1930) consider that the urea clearance test is considerably more sensitive than Addis' ratio, the phenolsulphonphthalein test or the determination of the urea or the creatinine only in the blood. Bruger and Mosenthal (1932c and 1932d) are of the same opinion. Freyberg (1935) considers on the contrary that the water concentration test is more sensitive than the urea clearance test. Bjerring (1934) finds that urea clearance and creatinine clearance are of equal value and that the former is more suitable for clinical use than the latter alone, since the urea clearance test gives a general expression of the function of the glomeruli and the tubules.

Chasis and Smith (1938) investigated the renal func-

tion in healthy and nephritic individuals by urea clearance and glomerular filtration determinations with inulin and came to the following conclusions: 1) No increased »back diffusion» of urea occurs in nephritic patients, as was considered by Rehberg (1926) to take place in certain cases.

2) The resorption of urea in nephritics takes place in principle in the same way as in healthy individuals.

3) The permeability of the tubular epithelium is unchanged in nephritis.

These statements have, nevertheless, been severely criticized by Ekehorn (1946) who showed from Chasis' and Smith's graphs that in actual fact the reverse condition occurs in nephritic patients; the more severe the degree of renal impairment the more urea is reabsorbed in analogy with the process in a normal kidney with a decreasing flow of urine.

These investigations will be discussed later in Chapter VIII, General Discussion.

### Clearance Investigations Suitable for the Determination of the Volume of the Glomerular Filtrate

As was mentioned in the foregoing (p. 4), the general clearance formula:  $\text{Clearance} = \frac{U \times V}{P}$ , where U is the concentration in the urine, V the volume of urine per minute and P the concentration in the plasma, is used under certain conditions for calculation of the volume of the glomerular filtrate. The following conditions must be fulfilled if the clearance of a certain substance is to equal the volume of this filtrate and be suitable for its determination.

1. The substance in question must be freely soluble in the plasma and filtrable through a semi-permeable membrane.

2. It must not be rediffused, reabsorbed, secreted or synthesized by the tubules in its passage through the kidney.

3. It should not be too difficult to determine in small amounts.

4. If it is necessary to use an exogenous substance, this must be atoxic.

It would be fairly simple if such a substance was normally present in the blood, and the character of the blood and the kidneys in these respects has therefore been the object of many investigations. Urea, which was originally considered to be a suitable substance, was later discarded since it was shown to be reabsorbed in the tubules. Thus still greater hopes have been based on another substance normally present in the plasma, i.e. creatinine.

### *Creatinine Clearance*

Creatinine is present in the blood plasma under normal conditions, according to Shaffer and Reinoso (1910) who showed that a positive Jaffé reaction similar to that obtained with diluted creatinine solutions occurs in protein-free plasma filtrate. Behre and Benedict (1922) considered, however, that creatinine is not present in the plasma in detectable quantities, but it must be considered as established by the investigations of Danielson (1936) and Miller and Dubos (1937) that the total chromogenic material present in the plasma is chiefly, if not entirely, composed of creatinine. This applies to the plasma, since 50-70 per cent of that chromogenic material which is present in the blood corpuscles is not comprised of creatinine (Hunter and Campbell 1917). By precipitating whole blood according to Folin's method (1930) the leakage of this non-creatinine chromogenic material from the erythrocytes is prevented (Miller and Dubos 1937). Shannon, Jolliffe and Smith (1932b) found that the concentration of chromogenic material in the plasma was sufficient to explain the normal excretion of creatinine in the urine, an opinion shared by Goudsmit (1936) who compared the concentration of chromogenic material giving a Jaffé reaction in the blood of the renal arteries and the renal veins.

Since the normal concentration of creatinine in the plasma is small, and a correct determination of it is difficult, attempts have been made to increase it by administering exo-



ogenous creatinine shortly before the clearance investigation. Rehberg (1926) who introduced creatinine clearance as a measure of the volume of glomerular filtration, considered that exogenous and endogenous creatinine are treated in an identical manner by the kidney. Smith, Finkelstein and Smith (1940) were of the same opinion but concluded that neither endogenous nor exogenous creatinine could be used to determine the volume of the glomerular filtrate owing to the fact that it is secreted by the tubules. Miller and Winkler (1938) maintain that the clearance of endogenous creatinine is identical with the glomerular filtration rate. This opinion is also shared by Steinitz and Türkand (1940). Berglund and Medes (1935) who investigated 38 healthy students without creatinine ingestion («endogenous creatinine clearance») and 60 after the oral ingestion of 3 g of creatinine («exogenous creatinine clearance») came to the following conclusions: The endogenous creatinine clearance is considerably lower than the exogenous, i.e. an average of 88.6 ml per minute compared with 172.9 ml per minute. The endogenous clearance nevertheless shows a positive correlation to the body weight whereas this correlation is lacking in exogenous clearance. They consider that in normal individuals it appears as though clearance of creatinine without ingestion gives an expression of the rate of glomerular filtration. After ingestion the filtration rate is increased for some considerable time, usually more than two hours, and this increase is probably due to the widening of the glomerular capillaries or to opening of inactive ones and a resulting increase in the blood flow.

Endogenous creatinine clearance is, however, very little used compared with clearance after the ingestion of creatinine, which is widely used (Holten and Rehberg 1929, Poulsson 1930, Ekehörn 1931, 1938, Ni and Rehberg 1930, Hayman et al. 1933, Bjering 1934, Cambier 1934, Gårdstam 1935).

Marshall and Grafflin (1932) nevertheless demonstrated that exogenous creatinine could be secreted by glomerular fish and this led Shannon, Jolliffe and Smith

(1932) to investigate the circumstances in the dog. They considered that they were able to show that creatinine is partly excreted through the tubules since its clearance is higher than that of xylose, which at that time was considered to be an exact measure of the volume of the glomerular filtrate. After administration of phlorhizin, which blocks the tubular activity, these two clearances approach each other. The condition was the same in the dogfish, *Squalus acanthias* (Shannon 1934). These «proofs» are nevertheless unconvincing, since Richards, Westfall and Bott (1934) and later Shannon as well (1935, 1936) established that xylose is reabsorbed in the tubules and that in the dog creatinine is not reabsorbed by them and its clearance is therefore identical to the volume of the glomerular filtrate. The latter fact was also demonstrated by Van Slyke, Hiller and Miller (1935) and Richards, Westfall and Bott (1936). The conditions are also identical as regards creatinine in the *Neoturus* (Bott 1946), the rabbit (Kaplan and Smith 1935) and the sheep (Shannon 1938).

Chasis, Jolliffe and Smith (1933) believed that they had shown that creatinine is also excreted by the tubules in man and Jolliffe and Chasis (1933) came to the same conclusion. They based their hypothesis on the fact that the clearance of creatinine is higher than that of xylose. With phlorhizin they were not, however, able to obtain any decrease in the creatinine clearance, either in relation to that of xylose or absolutely but instead brought about considerable glycosuria. Such proofs carry no more weight than do the earlier investigations on the dog carried out by the same workers and which they later revised (v. the foregoing, Shannon, Jolliffe and Smith 1932). Shannon (1935) reinvestigated these conditions in man using inulin as a comparison in place of xylose. He then found that at plasma concentrations of creatinine between 7.3 and 13 mg per cent the creatinine clearance was 30-40 per cent higher than that of inulin, whereas this difference decreased to 12 per cent when the creatinine plasma concentration was between 96 and 127 mg per cent. This was interpreted as a definite in-

dication of tubular secretion of creatinine. After the administration of phlorhizin the clearance of both substances was practically identical but definitely considerably lower than before phlorhizin. This was, however, explained as dependent on disturbances in glomerular activity caused by the toxic effects of the substance.

Findley (1938) is of the opinion that if 0.5 mg per cent is subtracted from the plasma creatinine concentration — i.e. approximately that quantity of chromogenic substance which he believes does not appear in the urine — the creatinine clearance will be constant at plasma concentrations between 1.6 and 13.8 mg per cent. He also believes that there are strong reasons against tubular secretion of creatinine. Abdon (1935) showed that the administration of creatinine causes the occurrence of a substance reminiscent of creatinine phosphoric acid and this complex could possibly be excreted by another mechanism than filtration (Rehberg 1938). The three forementioned investigations are discussed by Shannon and Ranges in a later paper (1941) in which they still maintain their belief in the existence of a tubular secretion of creatinine limited to a certain quantity per time unit.

The strange fact that creatinine is secreted by the tubules in man but not in any other mammal investigated led Smith and Clarke (1938) to investigate the conditions in anthropoid apes and other infra-human primates. They considered that they had furnished proof of the existence of tubular secretion in the orang-utan, the chimpanzee and the macacus. They found manifest differences in the orang-utan and the chimpanzee, but not in the macacus on which, however, few experiments were made. The conditions in the non-anthropoid apes are still obscure.

Ekehorn (1944) made a new critical investigation of the creatinine secretion on the basis of the forementioned work by Shannon and Smith et al. He is of the opinion that no proof of the tubular secretion of creatinine exists but that, on the contrary, it is the only substance that indubitably fulfills all the conditions necessary for a substance suitable

for filtration determinations. A discussion of these views is found in the summary at the end of this chapter, since it is necessary to discuss inulin clearance before tackling these problems. The normal figures for creatinine clearance are also given there.

### *Inulin Clearance*

In their study of the tubules of amphibia, Richards, Westfall and Bott (1934) sought some substance that, introduced into them at that site, was not resorbed actively or by diffusion. Amongst the substances they tested was inulin. It was found to be considerably less diffusible than creatinine or glucose, filtrable through the collodion membranes impermeable to protein, filtrable through the glomerular membrane of amphibia and not excreted by aglomerular fish after intravenous injection. In clearance investigations in the dog, its clearance was found to be equal to that of creatinine and no changes were found at varying plasma concentrations or flows of urine. Shannon and Smith (1935) and Shannon (1935) published an account of their investigations of inulin clearance in man and in the dog after having tested inulin independently of Richards et al. They came to the same conclusions, i.e. the clearance is independent of the plasma concentration, tested between 53 and 565 mg per cent (in the dog), phlorhizin does not affect the clearance either in the dog or in man, the clearance is unchanged even in a small urine flow with the resulting high concentration of inulin in it, which would contradict the theory of diffusion through the tubules (Shannon 1936). Van Slyke et al. (1935) were able to find complete agreement in their investigations in the dog and found, as did Richards' and Smith's school, that the clearances of inulin and creatinine are identical in this animal.

The inulin clearance is probably identical to the glomerular filtrate in *Squalus acanthias* (Shannon 1935), inulin and creatinine clearance identical in the frog (Forster 1938), in the *Necturus* (Bott 1946), the rabbit (Kaplan and Smith 1935) and the sheep (Shannon 1938). Richards,

Bott and Westfall (1938) found that there was no excretion of inulin in the frog if the tubules were perfused through the renal portal vein with a solution containing inulin, creatinine, uric acid and phenol red, whereas all the other substances were excreted. In the dog and the rabbit there was no excretion of inulin if the kidneys were perfused at such low pressure that no filtration could occur. On the other hand, phenol red and diodrast, which undergo tubular excretion, were excreted. Miller and Alving (1940) endeavoured to establish whether there was any tubular reabsorption of inulin by clearance determinations at high and low plasma concentrations. In six healthy individuals and six suffering from renal disease, the greatest difference between the clearances at high and low concentrations respectively was of the order of magnitude that at the greatest difference a maximal tubular resorption of 1 mg per minute, within the limits of the error of measurement, could be envisaged. These investigations, as well as a number of others with the same trend, are summarized by Smith (1943) in his »Lectures on the Kidney». He came to the final conclusion that all the evidence accumulated from the investigations on the various species indicates that inulin can suitably be used in determinations of the glomerular filtration rate. Ekehorn (1944) opposes this theory, since he believes that inulin, as do all other sugars, undergoes tubular reabsorption (v. Summary, p. 35).

Inulin is a polysaccharide of fructose and is found stored for nutritional purposes in the Dahlia, Cichoria and Jerusalem artichoke. It is poorly soluble in cold water but on heating it can be easily dissolved as much as to 20-30 per cent whereafter on cooling it once more precipitates after some hours. Inulin does not reduce Fehling's solution, is optically laevorotatory and, like starch and glucose, somewhat resistant to alkalis but easily hydrolyzed with acids. It is not split by any ferment in the organism but by some micro-organisms sometimes present in the urinary tract.

Inulin can, according to Haworth, Hirst and Percival (1932) be represented stereochemically as a chain of

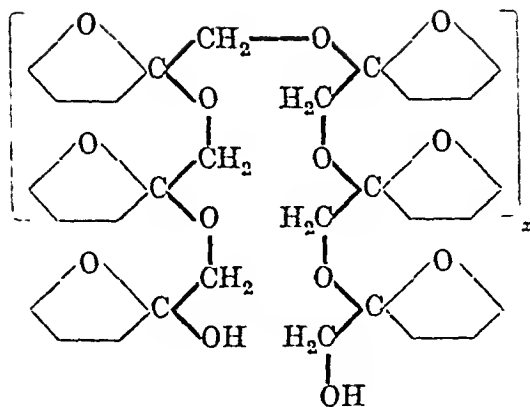


Fig. 1

fructofuranose units with a minimum length of 30 fructofuranose residues (fig. 1).

The molecular weight is given by Irvine et al. (1933) as 1700-1900, by Drew and Haworth (1928) as 3600-5200, by Berner (1930) as 3300-5100 and by Westfall and Landis (1936) as 4691-5615. The molecular weight of the Swedish inulin used by the present writer was determined by Örténblad (1943) as 3000-5000. The diffusion coefficient at 37°C (per sq.m and day) for cichoria inulin is 0.201 and for dahlia inulin 0.177 (Bunim, Smith and Smith 1937). There was no significant change in this coefficient after boiling in water or in 1 per cent solution of sodium chloride. It is lower than could be expected with regard to its molecular weight which is attributed to its elongate molecule. According to Spühler (1943) inulin is not bound to the plasma proteins, nor does it penetrate into the erythrocytes. It is not excreted in the gastric juice, the bile or the cerebrospinal fluid. It remains in solution in the urine for 24 hours and is not split there under normal conditions (v. above). According to Goldring and Smith (1936) pyrogenic reactions in the form of nausea, fever, lumbar pain, herpes and anuria sometimes occur after intravenous injection of inulin. Boiling for 30 minutes in distilled water does not decrease its toxicity. Partial hydrolysis with dilute acid decreases this somewhat and complete hydrolysis with N/10  $\text{H}_2\text{SO}_4$  gives a considerable decrease in toxicity. All pyrogenic

substance is, however, removed by filtration in a Seitz E.K. filter (Co Tni 1936, Smith, Chasis and Ranges 1938).

After the introduction of suitable and sensitive methods for quantitative determination of inulin, considerable interest has been aroused in the problem of inulin clearance (Smith et al. 1938, Berdal 1940, Røjel 1942, Jensen 1942, Hogeman 1943, Josephson and Lindahl 1943, Monstgaard 1945, and others).

*The Clearance of Other Substances Considered Suitable for Determination of the Glomerular Filtration Rate.*

*Sulphate and phosphate:* Mayrs (1922) was of the opinion that sulphate, phosphate and creatinine are concentrated to the same extent in the kidney in the rabbit. White (1923) found partial secretion of sulphate in the dog. Pouls-son (1930) estimated the clearance of creatinine and sulphate to be approximately identical in man, but his method is criticized by Cope (1932) who found that the sulphate clearance is only about 30 per cent of that of creatinine. According to Bjering and Øllgaard (1939) the clearance of «endogenous» sulphate is considerably lower than that of creatinine but this increases considerably in healthy individuals after the administration of sulphate, although it does not reach the level of creatinine clearance. In nephritic patients there is, however, no increase. White and Monaghan (1933) state that there is always a higher sulphate clearance at high plasma concentrations but that the phosphate clearance is in no way proportional to the plasma phosphate concentration.

*Ferrocyanide:* Van Slyke et al. (1935) found in the dog that the sodium ferrocyanide clearance is always identical to that of inulin and creatinine. Miller and Winkler (1936) demonstrated that in man the rate of ferrocyanide clearance is always the same as that of urea and thus undergoes tubular reabsorption to approximately 40 per cent. Moreover, this substance has a toxic effect on the human kidney.

*Cyanol* was considered by Höber (1930) to be a suitable substance for filtration determinations but according to Cope (1934) this is not the case, since its clearance is lower than that of creatinine and inulin respectively, probably mainly because it is bound to the plasma proteins.

*Creatine* and *hexamethenamine* are also less suitable for filtration determinations (Pitts 1934 a, 1934 b, 1936) and this is also true of *uric acid* (Bonsnes, Dill and Dana 1944) and *amino acids* (Kirk 1936).

Jolliffe, Shannon and Smith (1932 a, 1932 b) introduced the use of non-metabolized sugars, *xylose* and *sucrose*, but after thorough testing for more than a year in various species it was found that they were nevertheless reabsorbed by the tubules. It was therefore necessary to discard their use. Smith, Finkelstein and Smith (1940) later demonstrated that the non-metabolized sugars *sorbitol*, *mannitol* and *dulcitol* have the same clearance as inulin (and creatinine in the dog and the two first-mentioned in man as well and it would therefore be possible to use them for filtration determinations. According to Berger, Farber and Earle (1947) however, mannitol, which is the most frequently used, probably undergoes metabolic changes after injection in man, and must therefore be considered as unsuitable for such determinations.

### *Summary and Discussion*

As is seen in the foregoing, the majority of workers agree that inulin is a substance that is neither reabsorbed nor secreted by the tubules whereas it is freely filtrable in the glomeruli and thus well suited for filtration determinations. On the other hand, it is seen that creatinine, which was introduced by Rehberg as an ideal test substance, is unsatisfactory in several respects chiefly since it is considered to be partially excreted by means of an active tubular secretion. As mentioned earlier, this question has been investigated by Ekehorn (1944) who in a series of papers published in *Acta Medica Scandinavica* attacked the theory of renal function put forward by Homer W. Smith et al. Ekehorn



is of the opinion that he is able to refute the arguments put forward in favour of the secretion of creatinine and that the only remaining possibility is an active reabsorption of inulin by the tubules. A discussion of this matter must be based on the assumption that the following facts are established:

1. Both creatinine and inulin are freely soluble in the plasma and freely filtrable through the glomeruli (Shannon 1934, Hendrix, Westfall and Richards 1936).

2. In none of the species investigated was inulin observed to be secreted by the tubules or reabsorbed by them. (*inter alia* Shannon 1934).

3. The secretion of creatinine in man and in anthropoid apes is assumed by Smith and others, since its clearance in these species is lower at high plasma concentrations of the substance than at low concentrations. This should be due to the circumstance that at rising plasma levels a certain maximal amount of creatinine is gradually secreted by the tubules. In still further increment of the creatinine concentration in the plasma this small quantity of secreted creatinine affects the clearance in an increasingly low percentage. The clearance therefore falls and approaches that of inulin but never falls as low. This conclusion is based on the agreement of the conditions in substances definitely secreted by the tubules, such as phenol red and diodrast, in which the formentioned occurrence was found. This phenomenon, which is termed the »self-depression» of clearance, is therefore regarded by Smith and his school as a *definite* indication of tubular secretion.

Ekelhorn then demonstrated (1944) that in an investigation made by Smith and Clarke (1938) on a chimpanzee it can be calculated that, at a high plasma concentration of creatinine, no secretion of this substance can have taken place since the amount of creatinine secreted was negative (-3.8 mg per minute). According to Ekelhorn, the actual fact is instead that at high plasma concentrations of creatinine, the tubular wall cannot withstand the large amount of this substance filtered but that some part is rediffused through the tubules and the clearance therefore decreases under such

conditions. Support for this hypothesis is given by an investigation of Kay and Sheehan (1933) in which the same "self-depression" of clearance — or rather of a factor which is directly proportional to the clearance — was demonstrated in the rabbit in which Smith et al. consider that the secretion of creatinine is impossible since the clearances of inulin and creatinine are identical in this animal.

Ekehorn thus demonstrates that "self-depression" of clearance at high plasma concentrations of creatinine can be explained by the fact that the tubular wall cannot withstand the high concentrations of creatinine in its lumen. It is nevertheless difficult to understand how this fact can afford proof that a secretion of creatinine actually does not take place at low or high plasma concentrations. It is possible that secretion can occur in one segment of the tubule and diffusion in another. It is therefore not *a priori* impossible, even if not probable, that both processes occur simultaneously — or more correctly, that both processes play a rôle at different concentrations, thus secretion with low concentration and diffusion with high. Ekehorn, however, writes: "tubular secretion of a part of the urinary creatinine is clearly out of the question in man and apes. Therefore only the second alternative remains for explaining the difference between the creatinine and inulin clearances, that is to say, that a fraction of glomerularly filtered inulin is reabsorbed in the renal tubules of primates". No positive proof of this latter alternative is, however, given but Ekehorn's own reasoning speaks in favour of the actual occurrence of tubular secretion of creatinine at low plasma concentrations. If we consider Ekehorn's mode of calculation in the case of Smith's chimpanzee to be correct for high plasma concentrations (3.8 mg/min) this procedure should also be valid at low plasma concentrations. We then find a secretion of 2.8 mg per minute. It is moreover strange that in this experimental series we obtain no tubular secretion at still higher plasma levels where still higher minus figures would naturally be expected. The disputed figure could also be due to errors of measurement. This is, however, impossible

Table I NORMAL VALUES

## Inulin clearance

Author	Men		$\sigma$	Women		$\sigma$
	No. of cases	Clearance (ml pr min)		No. of cases	Clearance (ml pr min)	
Miller, Alving and Rubin (1940) .	5	115	16.4			
Friedman et al. (1941-47) .....	16	129	30.6	5	126	14.0
Foa, Woods, Peet and Foa (1942)	7	117	30.8			
Talbott, Pecora et al. (1942) ..	5	148	35.8			
Smith et al. (1943) .....	67	131	21.5	21	117	15.6
Hogeman (1943) .....	35	120	12.8			
Josephson and Lindahl (1943) ..	25	140	32.0			
Bradley (1947) .....	8	114	15.0	6	128	19.5
Berger, Farber and Earle (1947)	26	126	17.1	7	111	16.5
Total no.	194			39		

## Creatinine clearance

Author	No. of cases	Clearance (ml pr min)	$\sigma$
Holten and Rehberg (1931) .....	9	113-186	—
Lassen (1932) .....	21	159	55.3
Hayman, Halsted and Seyler (1933) .....	59	145	34
Berglund and Medes (1935) .....	60	173	33.6
Ekehorn (1944) .....	44	136	33.2
Dean and McCance (1947) .....	5	165	—
Total no.	198		

to state when we do not know the error of measurement of the method used. Ekehorn nevertheless considers this to be impossible, although in this case it is only the writers themselves who could give a satisfactory answer. However this may be, the figure pointed out by Ekehorn appears somewhat puzzling.

4. Tubular reabsorption of inulin is denied by Miller, Alving and Rubin (1940) who investigated inulin clearance at high and low plasma concentrations. If such reabsorption exists it should appear as an appreciable decrease in clearance at low plasma concentrations and be less apparent at high concentrations. Only in one of the 12 cases investigated (6 healthy individuals and 6 with renal impairment) was it impossible to exclude the possibility of a tubular resorption of maximally 1 mg per minute inclusive of the errors of measurement. Other workers, however, have found that inulin clearance is identical at high and low plasma concentrations.

It is therefore possible to state that there is reason to believe that inulin is neither secreted by the tubules nor reabsorbed — at any rate in measurable quantities. On the other hand, there is no direct proof that creatinine is not secreted through the tubules in man.

It remains to give the normal figures for inulin and creatinine clearances (Table I).

Ekelhorn's material given here is somewhat corrected with regard to the number of cases. In his publication the creatinine clearance for 341 healthy *subjects* was stated to be 135.7 ml per minute. Actually, in several instances a number of clearance periods in the same individual were counted as different individuals, e.g. 35 periods in *one* and 41 periods in *one* other individual were given as 79 cases. Obviously, when computing means in normal individuals one cannot use several determinations on some patients and only one from each of the others. The former patients would then have too much influence on the mean. In addition, patients with other diseases (tonsillitis, acute and chronic polyarthritis, hypertension, acromegaly, etc.) are excluded here as well as those over 50 years of age.

According to the foregoing table an appreciable difference exists in man between the inulin and creatinine clearances, although this is intrinsically of less importance from a purely practical viewpoint, since when changes in the rate of glomerular filtration occur it is probable that these changes in

clearance are proportionally of the same order of magnitude for both substances. It is of more significance that the normal values for inulin clearance show smaller variations than those of creatinine.

### Clearance Investigations in Order to Determine the "Effective" Renal Blood Flow and the Maximal Secretory Capacity of the Tubules

#### *Diodrast and Hippuran Clearance*

In the following table the name, composition and iodine content of the most common iodine contrast media are tabulated.

Table II

American name	Trade name	Per cent of iodine	Chemical name
Diodrast	Diodrast Diodine B Pyelosil B Pylumbriu B Perabrodil B Dijodon Umbradil	49.8	The diethanolamine salt of 3:5-diiodo-4-pyridone-N-acetic acid
Diodrast compound	Diodrast-compound	25	An aqueous solution of 40.5 per cent of diodrast and 9.5 per cent of 3:5-diiodo-4-pyridone-N-acetic acid.
Skiodan	Intron Iodairal Skiodan Abrodil B Tenebryl	52	Sodiummono-iodo-methane sulphonate.
Uroselectan	Iopax	42	Sodium 5-iodo-2-pyridone-N-acetate.
Neo-iopax	Uroselectan B Iodoxyl B Pyeloclon B Uropac B	51.5	Disodium-N-urethyl- 3: 5-diiodo-4-pyridoxyl-2: 6-dicarboxylate.
Hippuran	Hippuran Jodairal forte	33.8	Sodiumortho-iodo hippurate.

Of these substances, diodrast, hippuran and uroselectan have been shown to have the highest clearances (Smith and Ranges 1938). Only the two first-mentioned are more generally used and will therefore be discussed in the following.

Both diodrast and hippuran are white, crystalline substances, readily soluble in water and contain chemically bound iodine. The melting point of diodrast is 245-246°C and of ortho-iodo-hippuric acid 170-171°C.

As mentioned earlier (v. p. 11) Elsom et al. (1934) investigated the clearance of a number of organic iodine compounds and found the diodrast and hippuran clearances to be considerably higher than the simultaneous creatinine clearance in man and in the dog. They also found that at high plasma concentrations of these substances the clearance fell — a 'self-depression' occurred. This had earlier been observed in the dog as regards phenol red (Marshall and Crane 1924) and was later observed in man as well (Goldring, Clarke and Smith 1936). Since the clearance of a number of organic iodine compounds is still greater than that of phenol red, they are now used clinically instead of the latter substance. Landis, Elsom, Bott and Shields (1936) considered that hippuran clearance did not give more information about renal function than urea or creatinine clearance since the reduction of all three ran parallel in renal insufficiency. In 1937, however, other workers (Elsom, Bott and Walker) demonstrated that the hippuran clearance in the rabbit is practically as large as the renal plasma flow measured with Rein's thermotromuhr or Barcroft-Brodie's method. Smith, Goldring and Chasis (1938) made a closer study of diodrast and hippuran clearance in normal individuals. They pointed out that the greater the clearance of a substance the greater is the possibility for the kidney of entirely freeing the renal plasma from it during a single circulatory phase and its clearance thus approaches the flow of renal plasma. Since there is no reason to assume that all the blood that passes through the renal arteries is distributed to excretory tissue, it is not possible to calculate the entire renal plasma flow

by means of such a clearance, but only that effective for the occasion — »effective renal plasma flow». By dividing this plasma flow by the percentage of the plasma in the blood obtained from the haematocrit figure, it is possible to calculate the renal blood flow (R. B. F.). A substance suitable for such determinations must fulfill the following criteria:

1. It must have the highest possible clearance since this would then be nearest to the true flow of plasma.

2. The excretion of the substance must not be affected by any other substances present in the blood and which are excreted by the tubules since they could then interfere with the tubular excretion of the test substance.

3. Its plasma concentration must be low and below the »self-depression limit».

These conditions are fulfilled by diodrast and hippuran. As regards point 2, it can be stated that no substance has hitherto been shown to depress their clearance when simultaneously present in the blood. On the other hand, diodrast and hippuran depress the clearance of a number of other substances, such as phenol red (Smith et al. 1938), *p*-amino hippuric acid (Smith et al. 1945), penicillin (Rammelkamp and Bradley, 1943) etc.

To these criteria set up by Smith several can be added to allow of ascertaining the renal plasma flow from the diodrast clearance:

4. At low plasma concentrations the renal venous blood should be almost entirely free from iodine contrast or

5. if the red blood corpuscles contain some part of the contrast, this should be bound in such a way as to avoid the iodine contrast being delivered to the plasma during its passage through the kidney and thus offering the tubules more than is present in the plasma.

Direct investigation of the renal venous blood in man was earlier difficult to perform. Warren, Braunon and Merrill (1944) however, investigated the extraction of sodium *p*-amino hippuric acid by simultaneous determination of the arterial and renal venous blood by catheterization of the renal vein from one of the cubital veins via the right.

auricle using Forssmann's (1929) method. Diodrast has not been investigated in this way in man, but since the clearance of this substance and of *p*-amino hippuric acid are equally high, the forementioned investigation probably also elucidates the conditions regarding diodrast. The forementioned workers found an extraction of 0.85-1.00 (mean 0.90), thus the majority of the *p*-amino hippuric acid was eliminated during one passage through the kidney. The extraction was determined according to the formula:

$$\frac{\text{Conc. in the arterial blood} - \text{Conc. in the renal venous blood}}{\text{Conc. in the arterial blood}}$$

There can be several reasons for the small residue. All the blood had possibly not passed the excretory tissue, or part of the substance was possibly bound to the plasma proteins in such a way that it was not entirely freed in passage through the kidney, or the mechanism described in point 5 could be the cause.

It is easier to determine the extraction of diodrast in the dog than in man since it is possible in the former to examine the renal arterial and venous blood on explanted kidneys. According to White and Heinbecker (1940) the extraction is 0.74 and according to Corcoran, Smith and Page (1911) 0.84.

White, Findley and Edwards (1940) found that in normal individuals part of the diodrast penetrates into the corpuscles and that the ratio

$$\frac{\text{Diodrast per 100 cc arterial cell water}}{\text{Diodrast per 100 cc arterial plasma water}} = 0.32,$$

which corresponds to approximately 50 per cent of the quantity in the dog (White and Heinbecker 1940). The quantity of diodrast increment afforded by the cells is determined by a) the concentration of diodrast in the cells, b) the rate at which diodrast passes to or from the plasma at a decrease in concentration in the latter. Determination was made by observation of the ratio: cell diodrast to plasma diodrast at a falling concentration curve after intravenous



injection of diodrast. The exchange is rapid in the dog and the ratio is thus constant, but in man the quotient of the ratio rises from 0.32 to 1.20, thus indicating that diodrast is not eliminated as rapidly as the fall in the plasma concentration takes place.

White and Heinbecker (1940) calculated that in the dog approximately 13 per cent of the diodrast delivered to the tubules emanates from the blood corpuscles and that in this animal the diodrast clearance could therefore be 13 per cent higher than the renal blood flow. In man, however, the cellular concentration of diodrast is only 50 per cent of that of the dog. Moreover, the diodrast leaves the blood corpuscles considerably more slowly and it is therefore more correct to estimate the renal plasma flow by means of the diodrast clearance. There are thus several indications that diodrast clearance at low plasma concentrations affords a measure of the effective renal plasma flow. How low must this concentration be before »self-depression» occurs? White and Heinbecker (1940) consider 13 mg per cent to be the upper limit, whereas Corcoran et al. (1941) give 3-4 mg per cent for the dog. If the concentration is kept below 5 mg per cent in man there should be some measure of security.

By raising the diodrast concentration in the plasma the clearance becomes depressed, a fact that Smith and his school believe to be caused by a maximal tubular secretion then occurring. By determining this maximal secretion,  $T_m$ , at a high plasma level it is considered possible to obtain a measure of the amount of functioning excretory renal parenchyma. Diodrast- $T_m$  and diodrast clearance show as a rule a positive correlation (Goldring, Chasis, Ranges and Smith 1940) but under certain conditions the diodrast clearance can decrease although the diodrast- $T_m$  is normal. This is particularly applicable to such conditions in which changes in the tonus of the efferent arterioles are to be expected and are accompanied by decreased blood flow but unchanged excretory function of the tubules, e.g. effect of certain drugs (Goldring, Chasis, Ranges and Smith

1941, Smith 1943). In order to assess the significance of the diodrast clearance correctly, it is therefore considered safer to determine the diodrast- $T_m$  as well. If low diodrast clearance depends on tonus changes in the efferent arterioles,  $T_{mD}$  is normal; if, however, it depends on a reduction of the excretory renal parenchyma a proportional decrease in  $T_{mD}$  will also appear. By means of such combined tests it is now possible to obtain a picture of various changes in renal function in an entirely different way than formerly. From Ekehorn's statements (1944) that at high plasma concentrations of creatinine — when according to Smith and his school a maximal secretion of this substance takes place — part of it is rediffused through the tubules, there is reason to wonder whether the same occurrence cannot take

Table III Normal Values for Diodrast Clearance

Author		No. of cases	Injection	Clearance (ml per min)	Variation	Ranges of Variation	Sex
Chesley and Chesley	(1939)	30	Contin. infus.	567	459—818	389	F
White et al.	(1940)	11	Intrav. inject.	410	340—500	160	?
			Contin. infus.	517	405—670	265	
Black, Powell and Smith	(1941)	8	Contin. infus.	537	437—630	193	?
Friedman, Selzer and Rosenblum	(1941)	6	Contin. infus.	700	?	?	M
"	(1941)	5	Contin. infus.	661	?	?	F
Steinitz	(1941)	6	Contin. infus.	688	519—910	391	M
Smith	(1943)	17	Contin. infus.	677	323—993	670	M
Smith	(1943)	61	Contin. infus.	594	445—780	335	F
Hilden	(1943)	7	Intrav. inject.	411	367—464	97	M
Bruu, Kuudsen and Raaschou	(1945)	18	Subcut. inject.	585	439—691	252	M
Hilden	(1946)	12	Subcut. inject.	617	512—695	153	M
Hilden	(1946)	12	Subcut. inject.	608	530—740	210	F
Dean, McCance	(1947)	5	Contin. infus.	489	420—575	155	?

place as regards diodrast at high plasma levels. If this is the case, the possibility of using  $T_{mD}$  as a measure of the maximal excretory ability of the tubules is naturally lessened.

To sum up, it can be said that diodrast clearance at low plasma concentrations (below 5 mg per cent) in all probability furnishes a measure of the effective renal plasma flow. Moreover,  $T_{mD}$  possibly gives a measure of the excretory mass in the kidney provided that a part of the diodrast is not rediffused through the tubules at high plasma concentrations.

It appears from Table III that not only the mean figures vary (from 410 to 700) but that the variability also shows very great differences according to different writers, i.e. from 97 to 670. These differences can probably be due to different methods used and in part depend upon the fact that the series were sometimes small.

A question of great importance is whether any circulatory changes take place after injection of these highly concentrated iodine compounds and also whether there are any contra-indications for the test. It is known that a fall in the blood pressure and shock can occur after rapid injection of iodine contrast (Literature: v. Pendergrass, Chamberlin, Godfrey and Burdich 1942) but according to Goldring, Chasis, Ranges and Smith (1940) these do not occur after the ordinary »clearance doses». Edwards and Biguria (1934) found, however, that the blood pressure was affected in the dog and this and dilatation of the peripheral vessels was noted in the rabbit by Weatherall (1942). An investigation by Smith, Goldring and Chasis (1938a) in which the inulin and phenol red clearances were determined the first time simultaneously with diodrast and the second time with hippuran in the same eight normal individuals, shows that renal efficiency can also be affected in man. In the former test the clearances were 10-20 per cent higher than in the latter test. It is naturally impossible to say whether this was caused by an increase over the normal in the former or a decrease in the latter experimental series.

As regards contra-indications for the use of iodine compounds, both the literature and the instructions issued by the various chemical manufacturers usually advise great caution in any of the following conditions:

allergic conditions, particularly asthma.

pulmonary tuberculosis,

hepatic diseases,

renal diseases with a tendency to retention of non protein nitrogen.

Several cases of death after diodrast injections for urography are described. These fall into two groups: the first occurring almost instantaneously and interpreted as an allergic reaction; the second several days or weeks later of uraemia, when it was often considered that the iodine contrast had directly injured the renal parenchyma (Pendergrass, Chamberlin, Godfrey and Burdick 1942). If this were true, it would mean a very considerable limitation of the diodrast clearance method. Although certainly many hundreds, perhaps even thousands, of clearance determinations have hitherto been made in cases of renal insufficiency, there is no statement in the literature of a death following such an investigation. It is strange that death has followed a diodrast injection in urography but not in clearance determinations. The present writer (Hogeman 1948) pointed out, in order to explain this fact, that it is possible that the iodine contrast does not *per se* cause any injury to the renal parenchyma, but that the simultaneous compression used in urography with accompanying rise of pressure in the renal pelvis can cause further injury to an already impaired kidney with resulting retention of non protein nitrogen and uraemia. There is no direct evidence that the abdominal pressure actually injures the kidney, but such an occurrence does not appear improbable when the functional ability of the kidney is poor. This is naturally a difficult question to solve and it is possible that animal experiments could throw some light on the problem.

### *p*-Amino Hippuric Acid Clearance

In studying the clearance of certain substituted hippuric acid derivatives, Smith, Finkelstein, Aliminosa, Crawford and Graber (1945) found that *p*-amino hippuric acid and diodrast had the same clearance. They therefore recommended the use of the former substance for the following reasons:

1. *p*-amino hippuric acid does not penetrate into the red blood corpuscles *in vivo* and no increment from this source can occur during passage through the kidneys.

2. It is not bound, or only negligibly, to the plasma proteins.

3. It is easier to determine than diodrast (in the same way as the sulphha compounds).

The clearance of this substance is depressed by simultaneous injection of diodrast. These two clearances cannot therefore be determined at the same time.

The fact that the clearances of diodrast, hippuran and *p*-amino hippuric acid are identical gives further support to the theory that the limiting factor in the excretion of these substances at low plasma concentrations is the effective blood flow to the tubules rather than a limitation in the tubular excretory mechanism.

## Factors that Affect Renal Function

When the results obtained from the renal functional tests according to the methods described in the foregoing chapter are to be judged, it must be borne in mind that they can be affected by certain general factors. A short account will be given in the following of the effects caused by the age of the patient, the body temperature and certain anatomical conditions.

*Age.* Renal function changes with increasing age. Lewis and Alving (1938) investigated 100 men and showed that the renal function in normal men over the age of 40 exhibited the following appreciable changes: the urea clearance expressed as a percentage of the normal figure falls

from 100 per cent at the age of 40 to 55 per cent at the age of 89. The urea nitrogen in the plasma increases from 12.03 mg per cent to 17.62 mg per cent in the same period and the concentration capacity falls from 1.030 to 1.023.

Hilden (1946) investigated the diodrast clearance in 16 individuals between the age of 51 and 79 years (8 women and 8 men). He showed that at the latter age the diodrast clearance fell to 52 and 63 per cent respectively of the normal figure. He also found a decrease in the urea clearance, although this was less pronounced, so that the quotient of the ratio: urea clearance to diodrast clearance rises with increasing age. Here, as is usually the case, it is difficult to decide how much must be attributed to age alone and how much to the arteriosclerosis which always accompanies it to a greater or lesser degree. Furthermore, the material is rather too small to decide the influence of the age.

On the other hand there is some evidence that renal function at birth is considerably below that of the adult. Barnett, Perley and McGinnis (1940) determined the glomerular filtration rate in seven apparently full-term newborns and found that it was 20-40 per cent that of an adult. In a later publication (1942) they reported on the determination of inulin clearance in a 24-hour old infant suffering from exstrophy of the urinary bladder which considerably facilitated the collection of the urine. The filtration rate was 21 per cent of the normal figure in an adult. According to McCance and Young (1941) the inulin clearance in 3 infants, aged 6-13 days, was 43 per cent of the normal figure. In an investigation by Dean and McCance (1947) of 7 infants between 2 and 8 days old the inulin clearance was found to be 16.3-44.6 ml and the diodrast clearance 23.5-125 ml, both clearances calculated per  $1.73 \text{ m}^2$ . The same writers also found that in the newborn the creatinine clearance is identical with that of inulin and varies, although not in an exact proportion, with the urine flow per minute.

The present writer has not been able to deduce exactly from the literature how rapidly renal function increases after birth or at what age it achieves figures similar to those in

the adult. The few figures we have indicate that at any rate after 10-12 years of age the renal function is fully developed qualitatively and even quantitatively with the possible exception of the renal blood flow which is in relation to the minute volume and the total volume of the blood.

*Body temperature.* Farr and Moen (1939) investigated the urea clearance of seven rheumatic patients before and during heating in a hypertherm to 105° F (rectal temperature). Before the test the mean clearance was 105 per cent, during the period of rise in temperature 61.7 per cent and 75 per cent when the fever reached its maximum. They considered this fall to be due to a retarded blood flow to the kidneys, caused either by part of the blood flowing to the hyperaemic skin and thus less to the kidneys or possibly by some dehydration despite attempts to avoid it.

Smith et al (Smith 1943) also emphasized that changes occur in renal function in connexion with fever in pyrogenic reactions. These consist of a considerable increase in blood flow (measured by diodrast clearance) and a lesser increase in filtration (determined by inulin clearance) causing a fall in the filtration fraction (F.F.). This decrease in the F.F. is in turn attributed to a dilatation of the efferent arterioles of the glomeruli.

An entirely different explanation of certain changes in renal function can be given on the basis of the extremely valuable *anatomical* and *physiological* investigations of the circulation of the kidney published in 1947 by a research group in Oxford (Trueta, Barclay, Daniel, Franklin and Prichard: »Studies of the Renal Circulation»). They demonstrated that in the kidney of the rat and rabbit under certain experimental conditions (crush injury, after epinephrine and pituitrin injections, etc.) there is a shunting of blood from the cortex to the medulla through the juxta-medullary glomeruli and the vasa recta. This »by-pass» mechanism is favoured by the fact that the juxta-medullary glomeruli have considerably wider efferent arterioles than the cortical glomeruli and can thus permit a greater flow of blood. On normal aging and in various forms of Bright's

disease. the juxta-medullary glomeruli are easily subject to degenerative changes so that one glomerular loop is gradually dilated and takes over the entire blood flow through the glomerulus, the other glomerular capillaries becoming atrophied. The blood can consequently flow more easily through this passage and the longer and more difficult passage through the cortex is diminished. It is possible in this way to explain the decrease in renal function in increasing age as well as in certain renal diseases and changes due to shock.

Corcoran and Page (1943), Lauson, Bradley and Cournand (1941) and Selkurt (1946) studied the inulin and diodrast clearances during shock caused by haemorrhage in man and in the dog. They all came to the conclusion that in a fall in blood pressure to approximately 60 mm Hg. the clearance fell considerably or to zero, although on direct measurements some blood flow was found to be present (Selkurt 1946). After restoration of the blood pressure by blood transfusion the clearance figures did not immediately rise but remained at low levels for some time. They therefore concluded that clearance tests during and immediately after shock are of little value. The best explanation of the phenomena is given by the assumption of the 'bypass' mechanism according to Trueta et al. It is a controversial question whether the increase in non protein nitrogen often found in connexion with intestinal haemorrhages is to be attributed to the same mechanism or whether it is partly due to absorption of digested blood from the intestinal tract (Johnson 1940, Yuile and Hawkins 1941, Black, Powell and Smith 1941, Chunn and Harkins 1941). The decrease in renal function observed in cardiac insufficiency, particularly combined with intestinal stasis, (Lassen 1933, Seymour, Pritchard, Longley and Hayman 1942) is also most easily explained by this 'bypass' mechanism. Such circulatory changes and their possible significance in various renal diseases are discussed in Chapter VIII.

A purely anatomical detail that should be borne in mind in clearance determinations is pointed out by Gabriele



(1942). The renal artery usually originates at right angles to the aorta, but if this angle becomes obtuse or acute owing to a postural change of the kidney (asthenia, obesity, pregnancy, etc.) the conditions of pressure in the renal artery undergo a change. The filtration pressure and renal blood flow can thus change without any changes occurring in the kidney itself.

In the foregoing a short review of the modern theory regarding the renal function and of the tests worked out on the basis of this conception is given. For want of space it was impossible to enter into particulars and to evaluate the literature in this field.

### Some Terms and Definitions Used in this Paper

*Addis' ratio* =  $\frac{\text{Urea excretion, g per one hour}}{\text{Urea g in 100 ml of blood}}$  is a measure of the ability of the kidneys to excrete urea.

*Ambard's constant*:  $\frac{B}{\sqrt{D} \sqrt{\frac{U}{25}}}$  is also a measure of the

ability of the kidneys to excrete urea. B denotes the concentration of urea in the blood and U the concentration in the urine, both expressed in gm/lit. D denotes the quantity of urea excreted in 24 hours (in gm).

*Clearance* is defined as »the minimum volume of blood required to furnish the quantity of substance excreted in the urine in one minute's time» (Smith 1937). The ordinary clearance formula is written:  $Cl = \frac{U \times V}{P}$ . U and P are the concentrations of urea in the urine and in the plasma respectively and V the urine flow per min.

*Inulin clearance* is with all probability a measure of the glomerular filtration rate.

*Creatinine clearance* is probably somewhat larger than glomerular filtration since a small proportion of the creatinine is presumably excreted by tubular secretion.

*Urea clearance* is smaller than glomerular filtration since part of the urea is reabsorbed, a larger quantity with low than with high urine flow. It is usually stated as a percentage of a normal value instead of as ml/min.

*Diodrast clearance* is considered to be a measure of that amount of plasma that reaches the excretory tubular tissue. If this clearance figure is converted with the help of the haematocrit figure to whole blood, a measure of the so-called 'effective' renal blood flow is obtained. Diodrast is excreted both by the glomeruli and by the tubules.

$T_m$  is an expression of the maximal secretory or reabsorptive capacity of the tubules. The former is determined for example at a concentration of approximately 25-30 mg per cent of diodrast in the plasma and is denoted  $T_{ml}$  and the latter determined for example at a concentration of approximately 400-500 mg per cent of glucose in the plasma and denoted  $T_{mg}$ .

*Filtration fraction* = the quotient of the inulin and the diodrast clearances.

These explanations are intended to facilitate the reading of the present paper for those who are not earlier familiar with the terminology.

## The Problem

The modern methods described earlier, which allow the registration of separate functions of the kidney, afford possibilities of increasing our knowledge of renal function in healthy and diseased individuals under physiological and pathological conditions. The fact that the majority of earlier investigations have hitherto dealt with normal function and only a minority with the function occurring in renal disorders caused the present writer to apply these methods to a large clinical material. The chief reason why this has not been done earlier is probably the complicated technique used. The first task was therefore to simplify this technique in order to make it more suitable for routine investigations with the least possible inconvenience to the patient.

The following problems were then considered:

1. Does combined functional tests with determinations of the inulin and diodrast clearances give more information about renal function in persons with diseased kidneys than the simpler methods used earlier?

2. Does diodrast clearance, which is the most difficult test technically, together with determination of the glomerular filtration rate give more information than the latter test alone?

3. Is inulin to be preferred to creatinine in filtration determinations?

4. Is it possible with functional tests of this kind to make a better differential diagnosis and thus to obtain indications for therapy and prognosis? How far is it possible to judge renal function from clinical symptoms alone?

In the course of the investigation two further problems arose.

5. Is it possible following this investigation to draw any conclusions regarding the reserve capacity of the kidney?

6. Are there any possibilities of judging the manner of work of the kidneys when their function is failing?

In order to answer these questions a sufficiently large material is required which in certain cases must be followed up for some time. The investigation has thus been extended to several years and it was therefore not always possible, if the continuity was not to be disturbed, to make use of any newly-published modifications of the methods.

## CHAPTER II

# METHODS USED IN THE PRESENT PAPER

### Analytical Methods

The customary clinical methods were mainly used in this investigation and a detailed account of them is therefore superfluous. Some determinations of a more complex nature were nevertheless made in which the writer introduced certain modifications. A rather lengthy account of the technique of the latter therefore appears to be justified.

### Quantitative Iodine Determination

When quantitative determinations are to be made of diodrast, hippuran or other iodine-containing contrast media, all the methods are based on the determination of the iodine which is present in a fixed percentage in all these media (v. Table II p. 40). As regards diodrast, which is a pyridine iodine compound, several simple methods have been published recently (White and Rolf 1940 a, 1940 b, Alpert 1941). When it is a question of other iodine compounds than those containing pyridine iodine, more complicated and lengthy methods are necessary. The writer, who during the war was unable to obtain any pyridine iodine compound (diodrast or the corresponding German substance per-abrødil or uroselectan) was therefore in the first two or three years obliged to use hippuran (Swedish substitute: Hippodin Leo) which according to American publications (Elsom et al. 1934, Smith et al. 1938) has a clearance approximately the same as that of diodrast. After the end of the war the

writer changed to diodrast (Swedish substitute: Dijodon Leo) but nevertheless carried out his iodine analysis in the same way as previously.

After studying and carrying out preliminary tests with various methods, the writer chose a modification of Elmer's (1938) micro-iodine method. After thorough testing and lengthy elaborations this proved well suited to the purpose.

The principle of the method is: combustion of all organic matter in the presence of an alkali, concentration of the iodine and titrimetrical determination with thiosulphate.

- Reagents:* 1)  $K_2CO_3$  (reagent grade) in 50 per cent solution  
 2) Ethanol in 95 per cent solution  
 3) 0.5 N  $H_2SO_4$   
 4) Bromine water. 0.2-0.3 ml of bromine is added to 10 ml of redistilled water and shaken vigorously for a few minutes. This solution must be freshly made twice a week as otherwise  $HBr$  can be formed. It is kept in a dark bottle.  
 5) Starch in 0.5 per cent solution  
 6) Potassium iodide (reagent grade) in 0.5 per cent solution  
 7) 1/250 N  $Na_2S_2O_3$  (reagent grade). The solution is stabilized by the addition of 1 per cent amyl alcohol according to Reith and van Dijk (1937).

All the reagents were produced iodine-free according to the instructions given by Elmer (*»Iodine Metabolism and Thyroid Function»*, 1938, pp. 46-50).

### *Method*

I. 2 ml of plasma or urine is measured in a nickel crucible and mixed with 2 ml of  $K_2CO_3$  in 50 per cent solution. The mixture is then evaporated on a water bath to a thick pasty consistency (plasma) or dryness (urine) and thereafter placed in an electrical oven at  $160^\circ C$  until the sample is completely dried (40-50 minutes).

II. Combustion. The plasma sample is combusted for 45 minutes at  $420-430^\circ C$  whereas only 30 minutes at  $300^\circ C$  is required for the combustion of the urine. This difference in the temperature and the time necessary is due to the fact that a considerable amount of organic substances is present in the plasma, but almost entirely absent from the urine sample which is diluted 20-100 times. This fact is not pointed out by Elmer and it therefore took the present

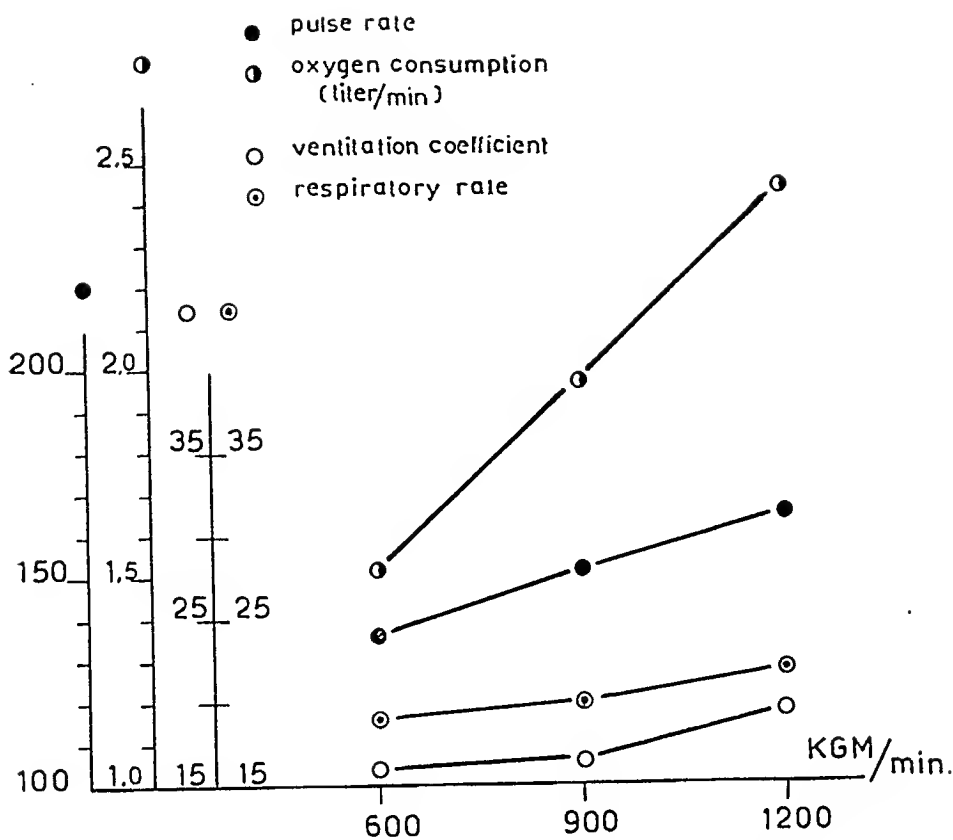


Fig. 5. Pulse and respiratory rates, ventilation coefficient and oxygen consumption at the various loads in a normal subject.

definite abnormal reactions exist in the groups. Compare for instance the pulse rate differences in Table 7, with the working capacities in Table 22 A in groups 4, 5, 7, 8 and 10.

The results in Tables 7 and 17 show that marked differences are found in groups containing cases which in advance could be regarded as less capable of work when compared to ordinary subjects. This is in accordance with the results of other investigators on the reactions of different functions to work. In most tests it is however not possible for a more able subject to distinguish himself above other subjects than those that are definitely abnormal. When for instance pulse or respiratory rates are used at a single light load as indicators of working capacity, nothing is known about the reaction at higher loads. The only possibility

approximately equal parts of redistilled water to prevent boiling in the crucible.

V. After careful evaporation a very small and quite white sediment is present in the bottom of the crucible, ready for iodine determination. This sediment is dissolved by adding 1.5 ml of hot redistilled water and cautious tilting of the crucible so that all the sediment, including any that may have fastened on the walls of the crucible, is dissolved. The solution is then poured into a small (25 ml) Erlenmeyer flask and the sides of the crucible rinsed twice with 0.5 ml of hot redistilled water. 3 to 4 drops of 0.5 N  $\text{H}_2\text{SO}_4$  are added to bring the pH to 1.5-2 (controlled by means of indicator paper and a platinum loop). Two drops of bromine water are added and the Erlenmeyer flask is placed on a sand bath with a temperature of 98-100° C for exactly 10 minutes to allow the excess of bromine to evaporate. Two or three times during this process the flask is lifted and the contents vigorously rotated once or twice to ensure complete evaporation. After 10 minutes all smell of bromine should have vanished and the liquid in the flask be absolutely clear.

VI. Titration. The flasks are cooled in cold water and 2 drops of 0.5 per cent potassium iodide solution added. A yellow or brownish-yellow colour then appears. Titration then takes place with 1/250 N  $\text{Na}_2\text{S}_2\text{O}_3$ . When the colour begins to fade, a few drops of starch solution are added and titration then continued until the blue colour vanishes entirely.

For calculation of the clearance, that amount of  $\text{Na}_2\text{S}_2\text{O}_3$  required for titration of the plasma and urine samples can be used as a measure of the quantity of iodine contrast (diodrast or hippuran) present in the samples, since the quantity of thiosulphate is obviously in direct proportion to the quantity of the former. If, however, an exact determination of the concentration of iodine is desired, a known quantity of iodine must be treated as described in Step V with  $\text{H}_2\text{SO}_4$  and bromine water, etc. This is then titrated and the amount of the thiosulphate solution required for this known quantity of iodine is then found. As an approximation it can be said that with the method of determination and the reagents used in the present investigation, 0.110 ml of thiosulphate solution corresponds to 10γ of iodine. Titration takes place in a 2 ml burette graduated in 1/100 of a ml. An accuracy of approximately 0.005 ml can be obtained with some practice.

### *Discussion of the Method*

Step I. An excess of alkali is added to the plasma and urine samples so that on combustion the freed iodine is bound in non-volatile form. NaOH can be used instead of  $\text{K}_2\text{CO}_3$ ,

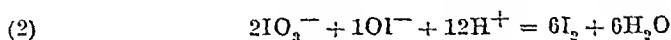
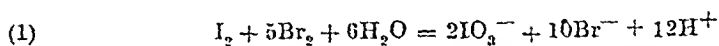


but the potassium salts are preferable since the paste formed after combustion and addition of water is then smoother and easier to extract than if sodium salts are used (Elmer 1938).

Step IV. Care should be taken to ensure that the ethanol used for extraction is of the strength prescribed. If it is too diluted the paste becomes thin in the course of extraction, thus making the procedure more difficult. If the ethanol is too concentrated, it is true that one can avoid more of other salts being dissolved in the extract, but the paste will then become dryer and dryer. If ethanol of the prescribed strength (94.2-95.2 per cent) is used, the consistency of the paste will remain unchanged throughout the extraction.

Step V. Acidification takes place in this method with  $\text{H}_2\text{SO}_4$ , but other acids can be used. The most important point to observe is that the solution must not be too acid, i.e. not below pH 1.5, as a loss of iodine then occurs.

The bromine water is added to oxidize the iodide to the iodate, the excess of bromine then being evaporated on the sand bath. Bromine water is more suitable than chlorine water, since the latter is both more difficult to produce and easily gives excessive figures for the iodine (Reith 1929). The absolute sensitivity of the method is increased on oxidation (the relation is naturally unchanged) as the amounts of iodine finally to be titrated are six times as large as those contained in the original material, according to the formula:



Step VI. Titration should take place in properly cooled solutions since the starch-iodine reaction is considerably more sensitive in cold than at room-temperature. Moreover, titration should be performed within one minute after the addition of the starch as otherwise an increase in the intensity of the colour will take place (Elmer 1938). If Nichol's starch reagent (with salicylic acid as a preservative) is used, the accuracy is still further increased (Turner 1930).

## The Accuracy of the Iodine Determination

In order to ascertain the sensitivity of the iodine determination method, the writer both made yield experiments and calculated the error of measurement by means of double determinations.

*Yield experiments.* These were made both on urine and on plasma. Varying amounts of a 3.7 mg per cent diodrast solution (calculated as iodine) were added to 2 ml of urine or plasma respectively. Each ml of this solution thus contained 37  $\gamma$  of iodine. Determinations were thereafter made in 16 single analyses for each concentration, 18.5  $\gamma$ /ml, 37  $\gamma$ /ml, 74  $\gamma$ /ml and 111  $\gamma$ /ml of urine and plasma. The results of these determinations are given in Table IV, in which the mean figure, the standard deviation and the deviation from the calculated amount expressed as a percentage are also given. It is seen that this deviation is approximately 1-1.5 per cent for the concentrations 37-111  $\gamma$ /ml. At concentrations of 74 and 111  $\gamma$ /ml there is throughout a small iodine loss both in the plasma and the urine tests. This does not, however, exceed — 1.6 per cent in the former and somewhat less (— 1.2 per cent) in the latter.

Table IV

Yield tests of diodrast in different known concentrations.

Concentration ( $\gamma$ pr ml)	Num- ber	$M \pm \epsilon(M)$	$\sigma$	Difference	Difference in %
Urine:					
111	16	$109.81 \pm 0.22$	0.87	— 1.19	— 1.1
74	16	$73.13 \pm 0.17$	0.70	— 0.87	— 1.2
37	16	$37.04 \pm 0.31$	1.22	+ 0.04	+ 0.1
18.5	16	$19.40 \pm 0.20$	0.81	+ 0.90	+ 4.9
Plasma:					
111	16	$109.23 \pm 0.64$	2.54	— 1.77	— 1.6
74	16	$73.56 \pm 0.41$	1.62	— 0.44	— 0.6
37	16	$37.32 \pm 0.34$	1.36	+ 0.32	+ 0.9
18.5	16	$19.82 \pm 0.28$	1.14	+ 1.32	+ 7.1

It is found throughout both in urine and plasma tests that with small additions of iodine the yield is too large whereas with additions of medium quantity the average values are fairly correct. With large additions of iodine the values are too low. This systematic deviation can, at least in part, be caused by the fact that the normal iodine concentration in the blood and the urine has been disregarded. Even if this is very small, its effect should be exactly that just described. It is natural for the systematic error calculated as a percentage to be relatively large in low figures. These yield experiments nevertheless show that the method is fairly accurate. Double determinations were carried out in order to ascertain this accuracy.

*The error of measurement.* These calculations are based on 193 double determinations of the diodrast in the plasma and 288 double determinations of its concentration in the urine. The material was divided into groups from 0-12.9, 13-25.9, etc. The classifications were chosen in view of the mean concentrations in the plasma in the normal material, which varied between 0.67-1.34 mg per cent (= 6.7-13.4  $\gamma$ /ml).

The error of measurement ( $\sigma_i$ ) is computed from the differences ( $d$ ) between double determinations according to the formula:

$$\sigma_i = \sqrt{\frac{\sum d^2}{2n}} \quad (\text{see Dahlberg, 1940})$$

Tables V and VI show the mean figures, the errors of measurement and the errors of measurement expressed as percentage values at various levels of the iodine concentration in the plasma and urine respectively. Here as well it is seen that the smallest percentage error is found in the concentrations of medium size whereas in low concentrations — and strangely enough in high ones — it is higher, the maximum being 8.4 per cent. This applies to the determinations in the plasma. The values are better and more even in respect of the urine determinations. The largest deviation is found with low values.

Table V

Error of measurement ( $\sigma_i$ ) of diodrast determinations, computed from double determinations on blood plasma.

Concentration $\gamma$ per ml	Number	Mean	Error of measurement ( $\sigma_i$ )	$\sigma_i$ in per cent of the mean
0 — 12.9	4	9.28	—	—
13 — 25.9	44	20.42	0.93	4.55
26 — 38.9	40	32.03	1.34	4.18
39 — 51.9	27	44.40	1.67	3.76
52 — 64.9	22	58.27	1.00	1.72
65 — 90.9	18	75.68	1.03	1.36
91 — 116.9	18	101.90	7.17	7.04
117 — 194.9	13	140.82	6.01	4.27

Table VI

Error of measurement ( $\sigma_i$ ) of diodrast determinations, computed from double determinations on urine.

Concentration $\gamma$ per ml	Number	Mean	Error of measurement ( $\sigma_i$ )	$\sigma_i$ in per cent of the mean
0 — 12.9	10	10.51	0.37	3.52
13 — 25.9	47	20.08	1.21	6.03
26 — 38.9	58	31.76	0.89	2.80
39 — 51.9	32	46.34	1.28	2.76
52 — 64.9	17	58.85	1.54	2.62
65 — 77.9	22	71.30	2.04	2.86
78 — 90.9	21	83.50	2.50	2.99
91 — 103.9	15	97.09	1.64	1.69
104 — 116.9	13	110.23	2.00	1.81
117 — 129.9	13	124.42	2.92	2.35
130 — 155.9	19	140.38	1.98	1.41
156 — 194.9	10	171.16	3.65	2.13
> 195	11	298.12	7.30	2.45

Group 9 contains cases that had no symptoms or diagnoses that could place them in another group. 2 cases had signs of slight hyperthyroidism; they both had capacities of 900 kg-m/min. 16 cases were of the leptosome body type, all having abnormal working capacity; 10 cases 900 kg-m/min and 6 cases 600 kg-m/min.

capacities of 900 kg-m/min. The others were normal.

e): 2 of the cases with orthostatic reaction showed working capacity. point of non-ability can be determined and indicates the working other factors than cardiac output or ventilation. Nevertheless the with anginal symptoms. Their working capacity is limited by sudden development of heart pains after a short period of work at 600 kg-m/min. This constitutes a functional test for patients in this group (4 having 900 kg-m/min, one 600 and one 300 kg-m/min). This last capacity (300 kg-m/min) was not founded on pulse or respiratory analysis; the man had to stop because of the

An abnormal working capacity was found in 6 of the 9 cases in this group (4 having 900 kg-m/min, one 600 and one 300 kg-m/min). One case (45) was discovered at a hypoxemia test with 9% oxygen, and one had signs only after work.

d). These cases had one or more of the following electrocardiographic abnormalities and usually subjective symptoms of coronary insufficiency, low or inverted T-waves or depressed ST-intervals. One case (45) was discovered at a hypoxemia test with 9% oxygen, and one had signs only after work.

c). One case had a working capacity of 600 kg-m/min, the other was normal.

b). 6 cases with slight signs of intra-ventricular conduction disturbance (QRS > 0.10 sec, notching of QRS) all had normal intraventricular block of the Wilson type had normal working capacity.

As they were otherwise normal at the examination and had no heart trouble they were not excluded from group 16, and will therefore be discussed with that group.

In addition, 4 athletes in group 16 had abnormal PQ values. after moderate work than at rest.

within the error-limits of the apparatus. But as a rule the PQ time in healthy subjects is usually some hundredths of a second shorter

The last-mentioned method, with small modifications; was used in the present investigation.

*Reagents:* 1)  $\text{ZnSO}_4$  (reagent grade) in 10 per cent solution

2) 0.5 N NaOH

3) Diphenylamine in 10 per cent solution: 10 g pure diphenylamine (reagent grade) is dissolved in absolute ethanol to 100 ml. This solution can be kept in a dark bottle for at least a week.

4) Hydrochloric acid and alcohol mixture: 80 volumes of concentrated HCl (chemically pure, preferably reagent grade) is mixed with 112 volumes of 95 per cent ethanol. This solution can also be kept for about a week in a dark bottle, but then often gives a higher blank than if it is freshly mixed each day.

For each determination a solution of reagents 3) and 4) is prepared, consisting of 12 volumes of reagent 3) and 192 volumes of reagent 4). This then constitutes the completed diphenylamine, hydrochloric acid and alcohol mixture.

### *Method*

*Plasma I.* 0.2-0.3 ml of washed yeast is added to 2-3 ml of plasma (heparin or oxalate) and the sample placed in the thermostat at  $38^\circ\text{C}$  for 45 minutes for fermentation. It is then removed and centrifuged rapidly.

II. 1 ml of the supernatant fluid, 7 ml of distilled water, 1 ml of the  $\text{ZnSO}_4$  solution and 1 ml of NaOH are mixed, strongly shaken, allowed to stand for 10 minutes and then filtered or centrifuged.

III. 5 ml of the filtrate and 10 ml of the diphenylamine, hydrochloric acid and alcohol mixture are measured in a special hydrolysis ampoule. A milky precipitate then appears which soon dissolves on shaking. The hydrolysis ampoule is sealed.

IV. The ampoule is placed in a water bath for exactly 60 minutes. This should boil strongly the whole time and the water level in the bath should be constantly somewhat higher than that of the fluid in the ampoule. After boiling, the ampoule is cooled rapidly in cold water for a few minutes.

V. Reading off in the Pulfrich stufenphotometer, filter S 61, against a blank of water treated in the same way as the plasma sample except that it need not be fermented.

*Urine.* The determinations are carried out in the usual way, but the samples do not require to be fermented unless severe glycosuria is present. The urine is diluted to a suitable concentration before determination. With the injection volume used by the writer in the present

investigation (usually 50 ml of a 10 per cent inulin solution) it is suitable to dilute the urine 20 times if the urine flow reaches 3-4 ml per minute. With a smaller flow the urine is diluted 100 times.

### *Discussion of the Method*

*Step I.* Fermentation of the plasma sample is necessary to eliminate the glucose normally present in the blood since this reacts with diphenylamine and gives a blue colour, as does fructose. According to Jolles (1910), van Creveld (1927) and Dische (1929), however, the glucose-diphenylamine blue colour reaction takes place more slowly than the corresponding reaction with fructose. For example, plasma with 400 mg per cent of glucose gives no colour after boiling for 15 minutes whereas a definite blue colour appears with a fructose solution of 1 mg per cent. On further boiling, however, the glucose gives a colour which gradually becomes more intense. This fact was utilized by Corcoran and Page (1939) who used a boiling time of exactly 15 minutes but nevertheless, for safety's sake, fermented the plasma samples. In modifications of their method, fermentation has often been considered unnecessary since a blank before injection of inulin gives a very weak colour (Røjel 1942, Guckelberger and Sanz 1942, Josephson and Lindahl 1943). Fermentation is nevertheless necessary for exact determinations particularly if the blood sugar concentration is high or if changes in it can be expected in the course of the test.

The majority of workers use a suspension of yeast in which the water content is determined by haematocrit before fermentation. In calculation of the concentration of inulin in the plasma, the dilution taking place through this increment of water to the plasma must be calculated, which is a complicated procedure. The writer therefore used the following method: 0.2-0.3 ml of yeast suspension is first added to each tube in which fermentation is to take place and the tube centrifuged rapidly for a few minutes. The supernatant water is then poured off and the tube inverted at an angle of 60° for 20-30 minutes, when practically all the water

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flows down the sides of the tube. Before the plasma is added for fermentation the inside of the tube is dried off with blotting paper. The plasma is then added and the yeast mass at the bottom of the tube carefully stirred with a glass rod and mixed with the plasma. This method was earlier introduced by Corcoran and Page (1939). It was not possible to note any dilution of the sample in repeated tests with this method. Neither inulin solution fermented in this way nor unfermented showed any difference apart from the analytical error.

Chasis, Redish, Goldring, Ranges and Smith (1945) stated that on re-examination of the inulin determination method it was shown that from a solution of repeatedly recrystallized inulin (thus definitely fructose-free) a certain measurable quantity of the inulin was absorbed by the yeast and the yield was therefore too low. If both the plasma and the urine are fermented the sources of error are practically negligible. The present writer paid due attention to this fact but was not able to find any significant decrease in the inulin concentration after fermentation either with pure inulin solutions or with inulin added to the urine: the fermented and unfermented samples showed no perceptible differences.

Such an investigation with the method used here was more difficult to perform in the blood, since unfermented plasma samples gave such high blanks that a direct comparison with fermented samples was impossible (an unfermented plasma sample gives a blue colour corresponding to 6-10 mg of inulin). If, however, the inulin is not absorbed by the yeast in pure inulin solutions or in urine and inulin mixtures, there is scarcely reason to believe that this would be the case if the inulin was mixed with the plasma.

*Step II.* In the original method, precipitation of protein according to Fujita and Iwatake (1931) with  $\text{CdSO}_4$  and NaOH is prescribed. Owing to the difficulty of obtaining cadmium sulphate during the war, and since this precipitation method is cumbersome, the writer was forced to use another method. Somogyi's (1930) precipitation with zinc



hydroxide, which is easy to carry out, was therefore chosen. According to Alving, Rubin and Miller (1939) tungstic acid cannot be used since a precipitate occurs on boiling, neither are trichloroacetic acid or mercuric chloride satisfactory as the blanks are too high.

*Step III.* According to the original method, heating in the water bath should take place in sealed glass vessels, and Alving, Rubin and Miller therefore used specially constructed test-tubes which, however, were unobtainable in Sweden. The writer therefore tried out glass tubes stoppered with many different kinds of rubber corks without success since the solutions became opalescent and caused very varying results. The reason for this is unknown, since Røjel (1942) and Laake (1945) were able to use this procedure and did not mention any disturbing opalescence. The writer instead used ampoules of Pyrex glass with a volume of 30 ml and provided with necks 20 cm long. After filling them, the extremity of the neck was sealed by melting. After boiling they were opened with an ordinary ampoule file. It was possible in this way to use the ampoules about ten times and when the «neck» was used up a new one could be melted on at very low cost. A similar procedure appears to have been used by other workers (Jensen 1942).

*Step IV.* The boiling time in sealed ampoules can scarcely be brought lower than 60 minutes as otherwise the hydrolysis may be incomplete (Hogeman 1943). Jensen (1942), who used propanol in a mixture of diphenylamine and hydrochloric acid instead of ethanol, since the former — owing to its higher boiling point — evaporated less than the latter and the risk of explosion of the tube is therefore diminished, also found a boiling time of 60 minutes most suitable. It is true that he found an increased colour intensity in the inulin sample if boiling was continued beyond this period, but this can be explained by the fact that after 60 minutes the blank assumes a more intense colour in the same proportion, so that the figures read off against it then become constant.

*Step V.* Reading-off takes place in a Pulphrich stufenphotometer since the photoelectric colorimeter, prescribed by the

original writers, was unobtainable. Either filter S61 or S66 can be used since the maximum light absorption of the blue colour formed lies at  $630\text{ m}\mu$ . The samples should be read off within 15 minutes as otherwise precipitation of the pigment gradually takes place, particularly if the concentration is high. Moreover, the colour slowly begins to fade after this time (Laake 1945).

### The Accuracy of the Inulin Determination

In the case of inulin, no yield experiments in the usual sense were made since such experiments have been made earlier by a number of workers (Corcoran and Page 1939, Alving, Rubin and Miller 1939, Spühler 1943, Laake 1945, and others). The present writer has, however, carried out a series of determinations in the urine and in plasma with varying glucose concentrations with the object of checking the method, particularly with regard to fermentation. In addition, the error of measurement was calculated on the basis of double determinations.

In the first experiments the writer used four different inulin solutions, with a respective inulin concentration of 220, 170, 110 and 51 mg per cent. 1:10 dilutions of these solutions were made with urine, normal plasma and plasma to which glucose was added to a concentration of 300-400 mg per cent, thus altogether three different series. The inulin concentration in these series was thus 22, 17, 11 and 5.1 mg per cent respectively.

The plasma samples in both series were fermented in the usual way for 45 minutes at  $38^{\circ}\text{C}$  whereas the urine samples were not fermented, in accordance with the method described (v. p. 65).

Two series were made with plasma in order to ascertain whether the fermentation process was sufficient even for high plasma concentrations of glucose.

The results of these experiments are seen in Table VII. The percentage error varied between  $\div 2.9$  and  $-3.4$  per cent, the values throughout being somewhat too low for plasma and slightly too high for the urine tests. Two factors can

Table VII

Yield tests of inulin in different known concentrations. Plasma A is plasma with 300 mg % glucose.  
 Plasma B is normal plasma.

Concen- tration (coeff. of extinct × 1000)	Plasma A				Plasma B				Urine						
	Num- ber	M ± s(M)	σ	Diff. in %	Num- ber	M ± s(M)	σ	Diff. in %	Num- ber	M ± s(M)	σ	Diff. in %			
630	16	626.9 ± 3.1	12.5	- 3.1	- 0.5	16	623.8 ± 2.7	10.9	- 6.2	- 1.0	16	634.3 ± 3.8	15.0	+ 4.3	+ 0.7
458	16	448.1 ± 2.5	9.8	- 9.9	- 2.2	16	450.0 ± 3.5	14.1	- 8.0	- 1.8	16	460.6 ± 2.5	10.3	+ 2.6	+ 0.6
290	16	285.6 ± 4.0	15.9	- 4.4	- 1.5	16	283.1 ± 1.8	7.0	- 6.9	- 2.4	16	290.0 ± 1.8	7.3	± 0	± 0
150	16	145.0 ± 1.8	7.3	- 5.0	- 3.4	16	148.8 ± 1.8	7.2	- 1.2	- 0.8	16	154.4 ± 2.0	7.9	+ 4.4	+ 2.9

contribute to these lower figures in the plasma tests. A certain small dilution can occur in the samples through the fermentation process, and the yeast can possibly absorb some part of the inulin, as Chasis et al. (1945) consider themselves to have demonstrated. The differences are nevertheless usually so small that these two factors cannot be considered to affect the usefulness of the method to any great extent.

*The error of measurement.* The calculations are based on 176 double determinations in the plasma and 178 in the urine. Fewer sub-groups were made here than in the iodine determinations, but the classification was also done here with regard to the normal level in the healthy individuals (expressed as the extinction coefficient  $\times 1000$ ).

Table VIII

Error of measurement ( $\sigma_i$ ) of inulin determinations, computed from double determinations on blood plasma.

Concentration: coefficient of extinction( $E_k$ ) $\times 1000$	Number	Mean	Error of measurement ( $\sigma_i$ )	$\sigma_i$ in per cent of the mean
0 — 239	11	206.1	7.6	3.69
240 — 479	68	353.7	8.1	2.29
480 — 959	85	682.3	9.75	1.43
$\geq 960$	12	1220.8	24.8	2.03

Table IX

Error of measurement ( $\sigma_i$ ) of inulin determinations, computed from double determinations on urine.

Concentration: coefficient of extinction( $E_k$ ) $\times 1000$	Number	Mean	Error of measurement ( $\sigma_i$ )	$\sigma_i$ in per cent of the mean
0 — 239	61	172.1	8.7	5.06
240 — 479	91	320.1	7.7	2.41
$\geq 480$	26	630.3	8.6	1.36

Tables VIII and IX show the mean figures, the errors of measurement and the percentage errors of the different groups. The percentage error in the plasma tests is 3.69-1.43 per cent and in the urine tests 5.06-1.36 per cent. In both groups of tests the percentage error is larger in low concentrations, which was to be expected.

### Quantitative Creatinine Determination

The present writer did not make a closer study of the various methods of creatinine determination but used that in current use at the University Hospital in Upsala (according to Rehberg 1926) based on Jaffé's colour reaction with picric acid in an alkaline solution. The determination is made on the urine or a suitable dilution of it (e.g. 1:2 or 1:10) if it is concentrated, and on plasma but not on haemolyzed blood. The reason that the blood must not be haemolyzed is that, as mentioned earlier (v. Chapter I) a chromogenic substance is present in the blood corpuscles that does not consist of creatinine and that on haemolysis can be absorbed by the plasma and give too high a figure for the Jaffé reaction. In uraemia too high creatinine figures can also be obtained with this method owing to the fact that certain retention substances, probably phenols, can interfere and give a positive Jaffé reaction. In routine determinations the error of measurement, according to the Central Laboratory of the Hospital, is about 8 per cent with low figures and still lower when the creatinine concentration rises above 15 mg per cent. The conditions are the same for determinations both on urine and on plasma.

### Quantitative Urea Nitrogen Determination

For this determination the writer also used the current method of the University Hospital in Upsala, i.e. that of W. Ohlson (1940). Its principle is: after precipitation of the proteins, the urea nitrogen in the blood is transferred by means of urease to ammonia bicarbonate. The nitrogen concentration is then determined colorimetrically after nesslerization.

The determination can be made both on plasma and on urine. The latter should, however, be diluted 10-20 times before the determination. After the writer had used this method for several years, its originator pointed out that the figures for the urine are usually too low unless plasma is added to the urine sample. He therefore recommended the following procedure: the determination is made both on 0.2 ml of plasma (or whole blood) and on 0.2 ml of dilute urine to which 0.2 ml of plasma (or whole blood) is added. In the former case the concentration of urea nitrogen is obtained directly by the formula given. In the latter case it is necessary to subtract the amount of urea nitrogen present in the plasma (or whole blood) added to the urine sample. The reason for this procedure is that otherwise too low a figure is obtained for the urine, probably owing to the fact that too few colloids are present.

Since the present writer was not aware of the facts in the foregoing until the majority of the determinations were already made, it was not possible to change the method for the remaining cases. The figures for urea clearance are therefore in all probability too low in relation to those of other authors. In the experiments that were later carried out to control the sensitivity of the method in its original form used by the present writer, the error was found to be approximately 10 per cent, somewhat higher in the urine than in the plasma determinations. The conditions are the same here as for creatinine determinations, i.e. the percentage error is proportionately greater at low than at high concentrations. The probable reason is that at low concentrations the colorimetry is more uncertain and the error in reading-off thus greater.

### Haematocrit Determination

The haematocrit determinations in the present investigation were carried out with the method later published by Nilsson (1948), i.e. in capillary tubes 50 mm long and with an inner diameter of 0.5 mm, placed in a specially constructed

centrifuge capsule. The centrifugal speed was approximately 6,500 revs/min and the time 10 minutes. Duplicate determinations were always made and the figure given for the respective cases was the mean figure. The differences, if such occurred, were not usually greater than one per cent. Heparinized blood was used, since it has been shown (Vahlquist 1941) that heparin does not affect the haematocrit figure.

### The Water Test According to Volhard and Strauss

Of the various water and concentration tests in use for the investigation of renal function, the writer chose the dilution and concentration test according to Volhard and Strauss (1927), since it has been the standard test for many years in the Medical Department of the University Hospital in Upsala and the hospital personnel are therefore familiar with it.

*Method.* No fluid is given after 8 p.m. on the evening preceding the test. On the following morning the bladder is emptied at 7 a.m. The body weight is noted. Between 7 and 7.30 a.m. the patient drinks one litre of water. The urine is then collected every 30 minutes between 8 a.m. and 12 noon and thereafter every two hours until 8 p.m. The night urine between 8 p.m. and 8 a.m. on the following day is collected in one vessel and the test is concluded by weighing the patient. No fluid other than the water given in the morning is allowed during the 24 hours of the test, but dry food is given. The portions of urine are measured and the specific gravity of each portion determined and corrected for temperature and possible protein in the urine sample. The volume and specific gravity of the respective portions of urine are entered in a table together with the following data: greatest dilution and greatest concentration; volume of excreted urine during the first two and four hours respectively, and the 24-hour volume. The patient is weighed in order to control the fluid balance of the body, dehydration, oedema, etc.

## Statistical Methods

In the statistical treatment of the material current statistical methods were employed.

The standard deviation ( $\sigma$ ) was calculated according to the formula:

$$\sigma = \pm \sqrt{\frac{\sum a^2}{n-1}}$$

where  $a$  designates the deviation from the mean and  $n$  the number of observations. When  $n$  exceeded 50, the standard deviation was calculated simply by using  $n$  instead of  $n-1$ .

The standard error of the mean,  $\varepsilon(M)$ , was calculated from the formula:

$$\varepsilon(M) = \pm \frac{\sigma}{\sqrt{n}}$$

The standard error of a percentage value,  $\varepsilon(P)$ , was calculated according to the formula:

$$\varepsilon(P) = \pm \sqrt{\frac{P(100-P)}{n}}$$

where  $n$  indicates the number of individuals and  $P$  is the percentage value.

The standard error of a difference,  $\varepsilon(D)$ , was calculated from the formula:

$$\varepsilon(D) = \pm \sqrt{\varepsilon^2(M_1) + \varepsilon^2(M_2)}$$

where  $\varepsilon(M_1)$  and  $\varepsilon(M_2)$  indicate the standard errors of the two series between which the subtraction was made.

The coefficient of correlation,  $r$ , was calculated from the formula:

$$r = \frac{1}{n} \sum \frac{a_x}{\sigma_x} \cdot \frac{a_y}{\sigma_y}$$

where  $a_x$  and  $a_y$  designate the distances of the  $x$  and  $y$  values from the arithmetical means of the respective series,  $\sigma_x$  and  $\sigma_y$  the standard deviations of the series and  $n$  the number of observations.

The standard error of the coefficient of correlation  $\varepsilon(r)$ , was computed from the formula:

$$\varepsilon(r) = \pm \frac{1-r^2}{\sqrt{n}}$$



## The Method Used for the Clearance Determinations

With the methods used by the writer it was possible to determine the inulin and diodrast clearances simultaneously, besides which in approximately 50 per cent of the material the urea clearance was determined at the same time and the creatinine clearance in about 50 per cent. Only in exceptional cases were these four clearance determinations made simultaneously, since it would have been impossible for one person to perform all these tests in one day as was necessary for technical reasons. The water test could obviously not be made on the same day as the clearance determinations but took place on the previous day or — more frequently — on the following. Reading of the blood pressure, haemoglobin determination and the blood counts were carried out either in the morning of the investigation or, if the figures had been constant for some time, on the day before or after the clearance determinations.

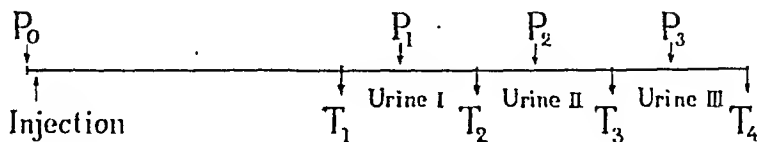
*Method.* The test is always made in the morning before the patient has eaten and is either in bed or in a half-recumbent position. Half an hour before the test, which is made at 8 a.m., the subject is given  $\frac{1}{2}$  to  $\frac{3}{4}$  of a litre of water to drink during this time. Healthy subjects or those only slightly ill are allowed to get up for urination. If there is any difficulty in urinating, catheterization is performed, preceded and followed by washing out of the bladder.

At 8 a.m. venipuncture is performed with the removal of 8-10 ml of blood for a blank test. All the blood samples are heparinized. The needle is allowed to remain in position in the vein and after removal of the syringe, the tube of a larger syringe is attached in which 50 ml of a 10 per cent solution of inulin and 10 ml of a 50 per cent solution of hippodina<sup>1</sup> (or dijonon) have previously been mixed. This is injected slowly (during 5-7 minutes). Immediately after the injection, 3 g of creatinine, dissolved in water, is administered orally if the creatinine clearance is to be determined.

After one hour the bladder is emptied, either spontaneously or, if necessary, by means of catheterization followed by washing out of the bladder with 50 ml of physiological saline solution. The time is noted. The bladder is emptied thereafter every 20 minutes three times, the time of urination being noted. If for some reason the bladder is not emptied at intervals of exactly 20 minutes, this is of minor importance provided that the exact time is known. Correction for the deviations in time can be made later. Exactly three minutes before the middle of each clearance period, venipuncture is made and 15-20 ml of blood withdrawn. Altogether three samples of venous blood are taken, exclusive of the blank. The samples are centrifuged and inulin, iodine and possibly creatinine and urea nitrogen determinations are thereafter made on the plasma. The volume of the urine is measured and the same determinations are made on suitable dilutions (v. Analytical Methods). The respective clearances are then calculated according to the customary formula:  $Cl = \frac{U \times V}{P}$

The calculations of the urea clearance are made according to the formula on page 21, i.e. the »maximum» or »standard» clearance is obtained.

*Diagram showing the procedure for the clearance tests*



$P_0$ - $P_3$  = venipuncture; the time is noted.

Urine I-III = the three consecutive urine samples with the time for the emptying of the bladder,  $T_1$ - $T_4$ , noted exactly.

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<sup>1</sup> The brand of inulin used was manufactured by A.B. Astra, Södertälje, Sweden, and the hippodin and dijonon by A.B. Leo, Hålsingborg, Sweden.

## Is it Possible to Use a Single Intravenous Injection for Clearance Determinations?

Since creatinine is administered orally and urea is already present in the plasma, this problem is concerned with inulin and diodrast<sup>1</sup> i.e. the two substances of which a single intravenous dose is injected at the beginning of the test.

Originally, continuous intravenous infusion of dilute inulin and diodrast solution was used and it was thus possible to maintain the plasma concentration of the respective substance at a desired level and practically constant during the time required for the test (Smith et al. 1938). This procedure nevertheless entailed catheterization followed by washing out of the bladder in order to ensure that it was completely emptied, thus necessitating the aid of two or three people in the performance of the test. A considerable simplification of the method for inulin determination was introduced by Alving and Miller (1940) who showed that the clearance could be calculated as well from a falling concentration curve after a single injection of inulin. Using this method, described by the present writer in the foregoing, it is usually possible to avoid catheterization and subsequent washing out of the bladder and the test can thus be made by one person. The method has therefore been used for the determination of inulin clearance with as satisfactory results as after continuous infusion (Alving and Miller 1940, Josephson and Lindahl 1943, Hogeman 1943, Ahlborg 1946, and others).

Conditions are somewhat different for the determination of diodrast (hippuran) clearance. Findley and White (1940), who considered the continuous infusion technique to be cumbersome and lengthy, used a subcutaneous injection of diodrast diluted 2-3 times with physiological saline solution in order to avoid local irritation and pain. Three patients were examined using this method and the diodrast clearance

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<sup>1</sup> In this discussion and in the following the term diodrast is used irrespective of whether diodrast or hippuran is referred to, in as much as these substances are excreted in an identical manner.

figures were then on the same level as that given by other workers after continuous infusion. They even made a control determination on one patient using the latter method and found the figures to be the same as after subcutaneous injection. Their tables, nevertheless, show that in all three patients the figure for the diodrast clearance in the third period (three clearance periods of 30 minutes each) was the lowest of the figures obtained. Hilden (1946) used the same method on a large material of healthy individuals, nephritic and hypertensive patients, making determinations for two clearance periods of 30 minutes each. The mean value for the diodrast clearance for 24 healthy individuals (12 men and 12 women) was in agreement with that found by other workers using continuous infusion. To the present writer it seems, however, remarkable that 22 of these 24 individuals had lower figures in the second than in the first period and only in two cases (9 per cent) were the conditions reversed. The iodine concentration in the plasma was between 2-0.6 mg per cent, judging from a graph showing the course of the concentration curves in eight patients (4 men and 4 women). The same conditions appear to be present in the patients with renal disease, according to Hilden's tables. The number of cases with a higher clearance value in the second period was, however, larger than in his normal material — of 173 clearance periods it was higher in 62, i.e. 36 per cent — but the majority showed higher figures in the first period. It should, nevertheless, be pointed out that the values in the majority of these patients were lower than those of the healthy individuals.

Josephson (1944, 1945, 1947) used intramuscular injections of diodrast and obtained clearance values in agreement with those obtained after continuous infusion. Of six healthy individuals, four showed the same fall in clearance from one period to another if those periods are counted that were taken 50 minutes after the injection and later when the plasma concentration curve definitely falls. The iodine concentration in these cases was between 5.52 and 3.5 mg per cent.

A fourth way of administering iodine contrast is by means of a single intravenous injection. As mentioned in the foregoing, this method has been used by the writer since 1942. The reason is that it is then possible to inject the contrast medium at the same time as the inulin and thus to avoid intramuscular or subcutaneous injection which is always accompanied by some trouble, tension or pain at the site of injection, discomfort in walking, etc. Following intravenous injection the patients usually suffered no discomfort whatsoever. Less than one per cent complained during the injection of general symptoms, vertigo, a sensation of heat in the neck or the abdomen or occasionally of nausea.

Single intravenous injections have also been used by other workers (White, Findley and Edwards 1940, Hilden 1943). With this method lower values were obtained throughout for the diodrast clearance than with other methods of administration (continuous infusion, subcutaneous or intramuscular injection). Moreover, the same circumstance can be observed after single intravenous as after subcutaneous or intramuscular injection, i.e. the clearance value usually falls from one period to the next, the highest figure occurring in the first and the lowest in the last period. In the present writer's investigations the plasma concentration of iodine in normal individuals was between 1.5 and 0.4 mg per cent.

The following two problems arise when a single intravenous injection of iodine contrast is used. 1) Why are the diodrast clearance values lower after this form of administration than after others? 2) Why is the clearance as a rule lower in each successive period after a single intravenous injection, a phenomenon that also fairly often occurs after both subcutaneous and intramuscular injections?

Since both these problems are intimately connected, they will be discussed together. As far as the present writer has been able to ascertain, the former question has only earlier been discussed in one publication, i.e. that of Josephson (1947). He found that in two of three healthy individuals investigated, the diodrast clearance was lower following intravenous injection than after intramuscular injection. He

also states: "however, the phenomenon with the lower diodrast clearance level, when stabilized after an intravenous diodrast injection, proved to be seldom occurring". He considered the explanation of this "depression" after intravenous injection to be either a spasm in the efferent arterioles with a decreased renal blood flow and an increased filtration pressure in the glomeruli, or a "back diffusion" of diodrast or "most likely" an inability of the exhausted tubular cells to clear the plasma from all diodrast present even when the concentration had fallen to a very low level. This last-mentioned hypothesis is, nevertheless, in part contradicted by some of Josephson's own experiments with combined intramuscular injection and a large intravenous dose of iodine contrast about one hour later. After the plasma concentration had fallen following the latter injection, the diodrast clearance value was the same as after the intramuscular injection "when the plasma concentration is never on a high level and the tubules can not thus have been previously exhausted". Another contradiction to the theory of tubular exhaustion is found in the fact that as regards creatinine, which probably is also excreted by tubular secretion, Shannon (1935) was able to demonstrate that no exhaustion mechanism existed. According to Shannon and Fisher (1938) the conditions are the same in respect of the reabsorptive capacity of the tubules for glucose.

During the writer's work with diodrast clearance determinations on falling concentration curves after a single intravenous injection, it was repeatedly found that the clearance value showed a most marked tendency to fall in each successive period in the case of healthy or practically healthy kidneys. The concentration of the iodine contrast in the plasma was between 1.5 and 0.1 mg per cent. In the case of persons with diseased kidneys, in whom several symptoms indicated impaired function, the diodrast clearance values did not show the same tendency to fall in each successive period. The differences in concentration for the mean plasma concentration in the various clearance periods were less and the concentration level in the plasma was higher than in normal

individuals. On the basis of these observations, the writer came to the following conclusion. Both the question of the lower clearance following a single intravenous injection and of the fall in each successive period at falling plasma concentrations (after intramuscular, subcutaneous or a single intravenous injection) are correlated and can be conditioned by three factors, i.e.

- A. The level of the concentration.
- B. The protein-binding of the iodine contrast.
- C. The fall in concentration *per se*.

That the level of the concentration can be of importance for the size of the clearance value is evident from the figures obtained after subcutaneous, intramuscular and single intravenous injections. If the concentrations are confined to below 5 mg per cent — above which level »self-depression» starts to occur and causes the clearance values to fall through this special mechanism — the figures are highest between 4 and 5 mg per cent and then fall in proportion to the decrease in the plasma concentration. This is illustrated by

Table X

Diodrast clearance ( $Cl_D$ ) and inulin clearance ( $Cl_{in}$ ) of 6 healthy human subjects simultaneously determined after one intragluteal injection of 20 ml 35 % umbradil (= diodrast). Length of periods about 20 min. The »peak» of the blood concentration curves coincides with the periods 2-3.

(Quoted from B. Josephson, Acta Med. Scand. 128 : 518, 1947).

Sex, age	♀ 34		♂ 34		♀ 26		♀ 36		♂ 27		♀ 36	
Period nr	$Cl_D$	$Cl_{in}$	$Cl_D$	$Cl_{in}$	$Cl_D$	$Cl_{in}$	$Cl_D$	$Cl_{in}$	$Cl_D$	$Cl_{in}$	$Cl_D$	$Cl_{in}$
1 .....	306	69	832	200	517	—	410	86	455	—	449	106
2 .....	645	91	787	105	545	76	468	86	747	76	720	88
3 .....	723	93	746	114	650	85	515	91	848	85	619	83
4 .....	510	72	727	133	328	47	490	90	825	79	613	66
5 .....	635	93	635	114	484	91	477	—	977	79	560	88
6 .....	449	72	486	103	557	83	479	94	988	83	438	74
7 .....	391	68	—	—	625	99	—	—	—	—	—	—

Table X which gives the clearance values in each period and the corresponding values for the inulin clearance of the respective individuals (taken from Josephson's 1917 publication). Each period is approximately 20 minutes, the first starting 10 minutes after the intramuscular injection. It is seen that in four of the six persons investigated, the first two periods show rising figures, possibly owing to the fact that

Cone

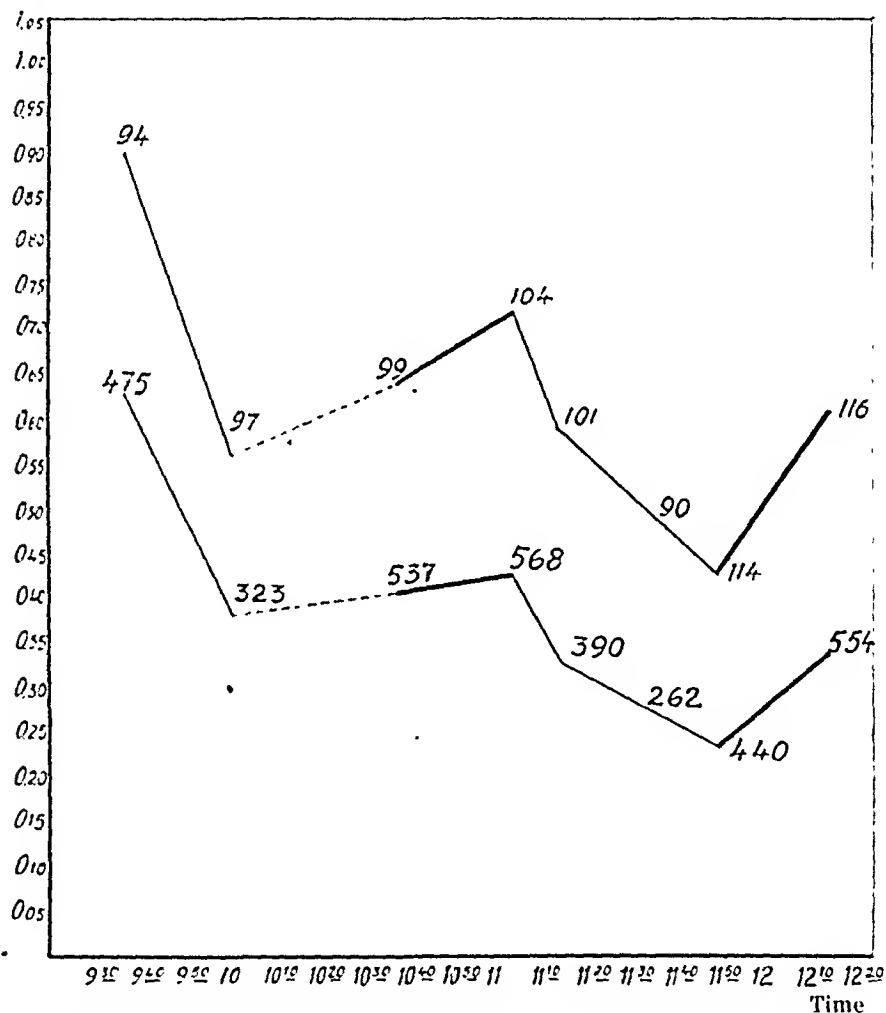


Fig 2. Diagram showing the values for inulin clearance (upper curve) and diodrast clearance (lower curve) at intravenous infusion (thick lines) and on falling concentration curve (thin lines). The figures indicate clearances in ml per min. The concentration of inulin in plasma is marked as the extinction coefficient, that of diodrast is obtained in mg % by multiplying the marked concentration with 5.



the »self-depression» exerts a successively decreasing influence. As a matter of fact the figures then reach a maximum and thereafter fall gradually as the plasma concentration falls.

In order to investigate whether the concentration level is of importance for the size of the clearance value, it is necessary to perform tests on the same individual with *constant* plasma concentrations at varying levels. No results of such investigations appear to be published. The writer therefore made such an experiment on himself (v. fig 2). 50 ml of a 10 per cent inulin solution and 10 ml of a 35 per cent diodrast solution were administered in a single intravenous dose. After 20 minutes two clearance periods of 20 minutes each were made on a falling concentration curve. Intravenous infusion was then introduced and the plasma

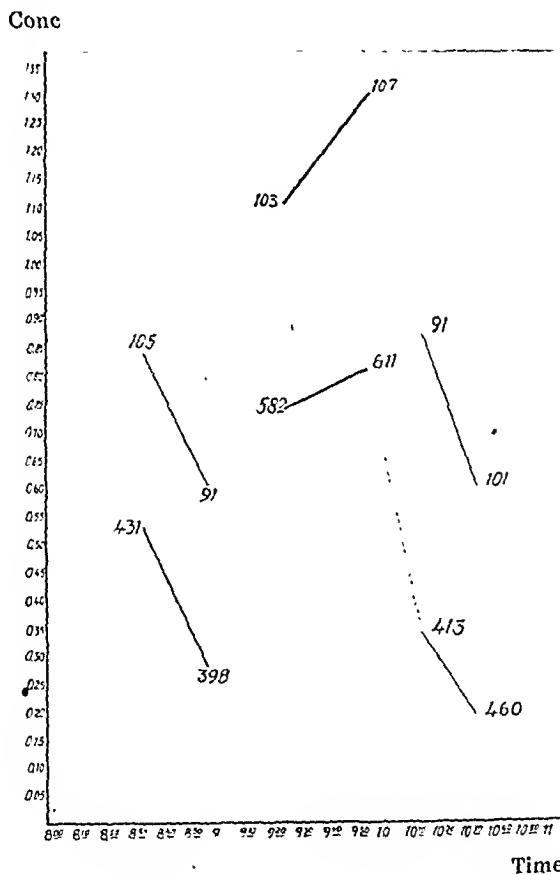


Fig 3. Diagram of the same kind as in fig 2.

Conc

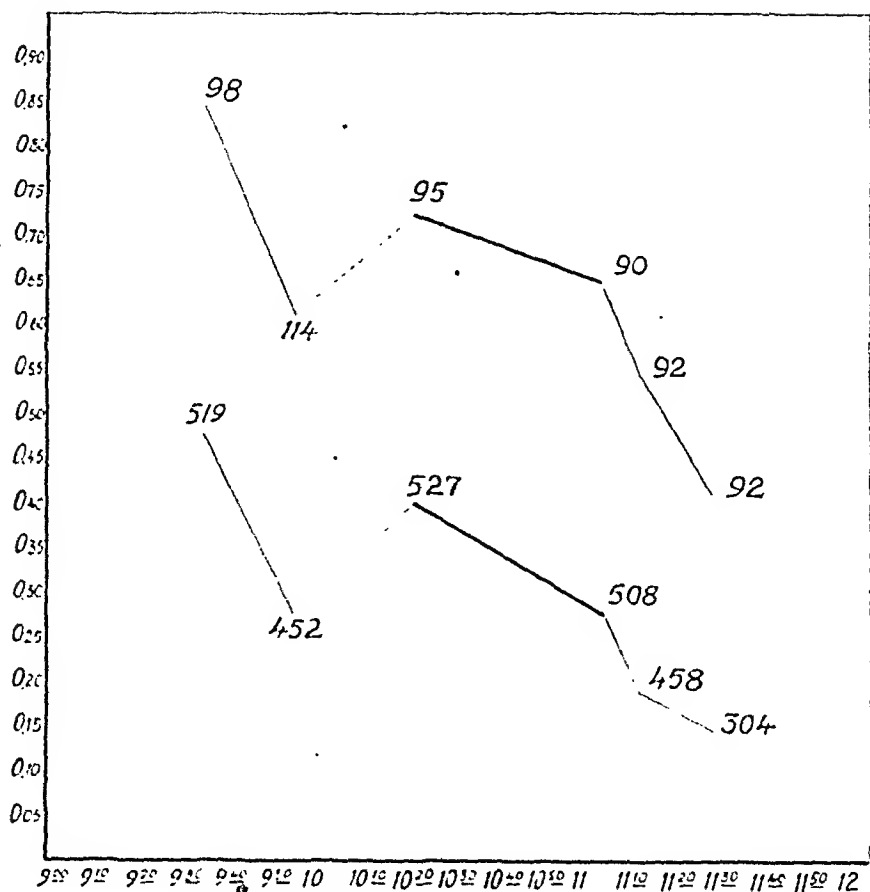


Fig. 3. Diagram of the same kind as in fig 2.

Time

concentration kept almost constant during 50-60 minutes. Two clearance periods were made during this time and the infusion stopped at the end of the second period. The plasma concentration was then once more allowed to fall for 40 minutes and two further clearance tests noted during this time. The infusion was once more introduced and the plasma concentration again kept almost constant for 40 minutes so that two further clearance periods could be registered. It was possible in this way to obtain clearance determinations on the same individual at two different, almost constant, plasma concentrations. The results are seen in fig 2 in which the plasma concentration curve is also drawn.

The experiment showed that the clearance values were

highest during the time of the continuous infusion and that they become increasingly lower with falling plasma concentrations. Moreover, it was observed that if the level of iodine contrast rises during the course of the infusion, the clearance value also rises.

Two other similar experiments were made after an initial single injection but the falling concentration curve was then only interrupted once by means of introduction of continuous infusion (figs. 3 and 4). In one experiment the concentration rose very slowly during this time and in the other it fell. Nevertheless, both experiments demonstrated that when a continuous infusion is introduced, the clearance rises and the absolute values depend on whether the iodine concentration in the plasma rises or falls during this time.

The three forementioned experiments were made after a single intravenous injection had been given and the concentration of iodine contrast had thus fallen from a relatively high level (15-20 mg per cent) to 1-2 mg per cent. Thus shortly after the injection, the plasma concentration was so high that the tubular cells performed a maximal secretion of the contrast. Despite this certainly short but nevertheless maximal output, no signs of «exhaustion» appeared. If continuous infusion was introduced and the plasma concentration of iodine was raised in order to obtain a constant level, the clearance value immediately rose. The experiments thus indicate that no exhaustion occurs in the secretory power of the tubular cells at least after a short maximal work and such a mechanism must therefore be considered as a less probable reason for the lower clearance values after a single intravenous injection.

There are thus three remaining possible explanations. One is that a spasm occurs in the afferent or efferent arterioles and that less blood therefore flows through the kidney at falling concentrations. Another is that the tubular cells require a certain time to adapt themselves to the output when the plasma concentration changes. The last possibility is that the whole blood is less effectively freed at falling concentrations of the iodine contrast.

The first possibility, i.e. a spasm in the afferent or efferent arterioles, can probably be excluded from the discussion on the following grounds. The inulin concentration and inulin clearance were determined simultaneously with the diodrast clearance in the three formentioned experiments and the inulin clearance then proved to be practically independent of the level of the inulin concentration, of a fall in it or its constancy. On the other hand, the filtration fraction changed so that it became consistently lowest when the plasma concentration of inulin and diodrast was maintained at a constant or almost constant level. This means that a spasm in the afferent arterioles could not occur, since the filtration would then fall to the same extent that the diodrast clearance fell. On the other hand, an increased tonus in the efferent arterioles at falling iodine concentration in the plasma could explain this phenomenon, but it would then be necessary, for this to increase more as the iodine level decreases. Were the conditions reversed, i.e. an increased tonus at increasing concentrations, it would be more understandable.

The second possibility, i.e. the existence of a »time factor» allowing the tubular cells to »adapt» themselves to the change in the iodine concentration in the plasma, is also difficult to accept. It is seen from the reproduction of the curves (v. figs. 2-4) that the diodrast clearance rapidly rises to a »normal» level if the concentration in the plasma is constant or nearly constant. This is, however, to a small degree dependent on the actual level of the concentration. It is true that if the plasma concentration is higher during continuous infusion (n.b. nevertheless always below 5 mg per cent) the clearance figure is higher. The differences are, however, considerably smaller than in the figures at falling plasma concentrations when no increment of iodine contrast is administered. Moreover, should a time factor be the only decisive factor in this fall in the clearance level, it should become less and less pronounced the lower the concentration, since at a falling concentration the curve for the concentration in the plasma increasingly approaches a horizontal line. The differences in the plasma concentration become

therefore successively smaller for each period and the clearance value should therefore become increasingly higher and not *vice versa*.

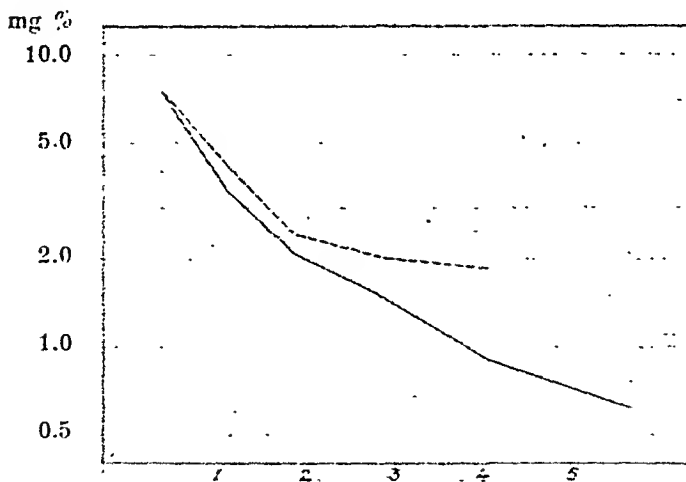
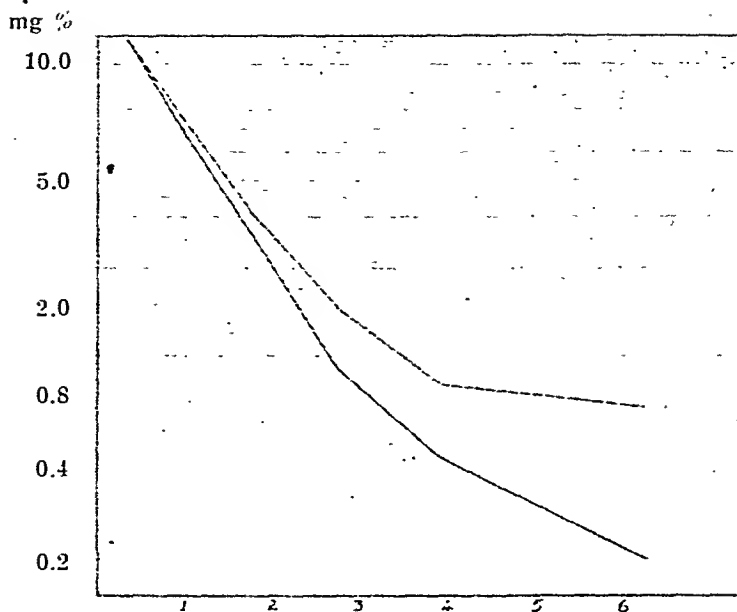
The third possibility is that the blood is less effectively freed from the contrast at falling concentrations. This in turn would depend either on the presence of part of the iodine contrast in the blood corpuscles, which would slowly deliver this substance to the plasma, or a certain quantity of the contrast being bound to the plasma proteins.

The first hypothesis was studied by White, Findley and Edwards (1940). They considered that some part of the diodrast penetrated into the red blood corpuscles and that the quotient of the ratio:

$$\frac{\text{diodrast per 100 cc arterial cell water}}{\text{diodrast per 100 cc arterial plasma water}} = 0.32$$

This quotient then increased at falling concentrations of the contrast in the blood. This was interpreted as depending on the fact that the diffusion of the contrast from the blood corpuscles to the plasma takes place slowly, and more slowly than the fall in the plasma concentration.

The present writer also investigated this condition in the venous blood at falling concentration curves but was unable to observe any appreciable increase in this quotient. It showed a mean figure of 0.33 in the determinations in eight healthy individuals at an iodine concentration of 3-5 mg per cent and rose to a mean figure of 0.34 in the same individuals after the iodine concentration had fallen to 1-2 mg per cent after 40 minutes. On the other hand, this investigation showed that part of the iodine contrast — approximately 30 per cent — diffuses into the blood corpuscles, but this can not explain why the clearance falls at falling plasma concentrations of iodine. The iodine concentration determined in the venous blood at a given moment is practically identical to that in the renal arterial blood at the same moment although, of course, there must be some difference. Since an equilibrium has already occurred in the relation between the iodine in the plasma and the iodine in the blood cor-



Figs. 5 and 6. Diagram showing the fall of concentration in solutions of plasma and diodrast (upper line) and of water and diodrast (lower line) of equal percentage during dialysis against physiological NaCl solution. The respective concentrations are plotted on a semi-logarithmical paper. Time in hours.

puscles, and the determination is made on plasma, there is no reason to assume that any further increment of iodine is delivered to the plasma from the corpuscles during the passage from the antecubital vein to the renal artery. If this were the case, the clearance should be higher when the blood concentration falls and not *vice versa*.

The possibility that the cause is the binding of part of the contrast to the plasma proteins is left. That this actually occurs has been shown by Smith and Smith (1938) who found that about 70 per cent of diodrast and 50 per cent of hippuran are thus bound. The present writer investigated this condition by means of simple dialysis experiments under the same conditions with a water and diodrast solution and with a diodrast and plasma solution of equal strength. The results are seen in figs. 5 and 6 (v. p. 89). It is seen from these diagrams that the fall in diodrast concentration is initially almost identical in both, but that at lower concentrations the fall in concentration in the plasma and diodrast solution becomes successively smaller so that the difference between the two curves becomes increasingly larger at lower concentrations.

The results of these dialysis experiments cannot, however, be applied directly to the conditions in the tubules — or rather in the border zone between the tubular cells and the surrounding capillary network. It is possible that the tubules excrete all the iodine contrast present in the blood — both that freely soluble and that bound to the plasma proteins — but it is also possible that the latter is not excreted, or at any rate only partially, during its passage through the kidney. Since that part of the contrast bound to the plasma proteins — judging by the dialysis curves — becomes proportionately greater the lower the concentration of the contrast in the plasma, this could naturally explain why the clearance falls at falling concentrations. In order to prove this theory, it would be necessary to investigate the clearance at falling plasma concentrations for substances having an identical clearance level but which are bound very differently to the plasma proteins. The present writer was not able to do this.

A difficulty in this discussion is nevertheless the fact that those workers who determined the iodine concentration in the plasma according to some method in which precipitation of proteins was included and the concentration was thus determined on *protein-free* filtrate; also found the same phenomenon, i.e. a falling clearance the lower the iodine

concentration in the filtrate. This can, naturally, be dependent either on the fact that this mechanism of binding plays no rôle in the problem of the falling clearance or that on precipitation of the proteins the protein-bound fraction is freed from the protein and joins the filtrate. The process of excretion through the tubules is certainly not comparable with the process in precipitation of proteins and our theories are therefore more or less confined to guesswork.

Should, however, the same process take place *in vivo*, it is hard to explain the decrease in diodrast clearance at falling plasma concentrations. Under such conditions there are only two courses open, i.e. to assume that the process takes place in another way *in vivo*, or to allow this problem to remain unsolved. Since, however, we are aware that protein binding takes place and that this occurrence can explain the phenomenon, the question of the activity of the tubular cells as regards protein-bound and freely soluble iodine contrast must be left open until definite information can be obtained.

The writer has thus endeavoured to discuss those factors that can be thought to condition the successive decrease of the clearance at falling plasma concentrations. His conclusion is that the most probable explanation is that this factor is the protein-binding of the iodine contrast, although this is not the only possible solution. Whatever the cause may be, it is nevertheless evident that this fall in the clearance occurs the more rapidly the fall in concentration takes place in the plasma. This decrease takes place more rapidly and is more pronounced in a healthy individual than in one whose kidneys are more or less impaired, when the excretion of the contrast is poorer, with a slower fall in the plasma concentration curve. This mechanism would thus be more effective in healthy than in diseased individuals and the figures for the former proportionately lower than for the latter had both been tested at constant plasma concentrations. In other words, the figures for the individuals with healthy kidneys are proportionately lower with this method (single intravenous injection and calculation on a falling concentration



curve) than for those with impaired kidneys and the differences between them consequently smaller than they actually are.

This can be considered as a moderate inconvenience from a diagnostic point of view. With regard to the present investigation, it would mean that the differences, if such should occur, would be minimum figures and thus more reliable than those in the figures obtained.

It is thus evident from the foregoing discussion that it is only possible to obtain clearance values that are constant from one period to another if the plasma concentration can be kept at the same level. Moreover, there is possibly a slight difference in the figures if the iodine concentration is maintained at a high level (i.e. near the »depression limit») or at a low level, but this difference is very slight. In the case of healthy individuals, a continuous infusion of standardized composition and rate of flow can be expected to maintain a fairly constant plasma level in the blood. If, on the other hand, a standard solution is administered at a constant speed and function is severely impaired, the concentration of the infused substances rises in the plasma and the clearance rises successively the greater the number of clearance periods. The only possibility is then to repeat the investigation at a lower concentration or with a slower rate of infusion. In ordinary clinical work this implies considerable difficulty, since the continuous infusion method is cumbersome and lengthy both for the examiner and for the patient.

What is the effect when subcutaneous or intramuscular methods are used? In both cases, after an initial rising plasma concentration, a »peak» in the concentration curve occurs and thereafter a successive fall. The height of this »peak» and the rate at which the concentration curve falls depend on the rate of absorption in that tissue in which the substance is injected and on the extent of renal function as well as on the quantity injected. If a clearance period is obtained in which the »peak» of the concentration curve coincides with the middle of this period, the clearance value will be maximal and rising towards this figure before the »peak» and falling

thereafter, (n.b. if the »peak» is below the »self-depression» limit). The majority of workers who have used the intramuscular or subcutaneous injection method have, however, waited 40-60 minutes after the injection before registering the first clearance period. The maximum of the concentration curve has therefore already been passed and the curve falls successively. This also explains why those who have used this technique have obtained successively lower clearance figures.

This tendency to lower clearance values in each successive period is naturally most pronounced after single intravenous injection, since the iodine concentration in the plasma then falls more rapidly than after subcutaneous or intramuscular injections, but *in principle* they are the same although they differ quantitatively. This also explains why, after an intravenous injection, the clearance values are generally lower than with other methods used. The workers who used the last-mentioned technique nevertheless consider the subcutaneous or intramuscular injection to be preferable, since it is then possible to approach more nearly those figures given by Smith et al. as normal values. In principle, however, there is no difference. It is also possible that after a careful study of this problem some correction factor might be obtained for the protein-binding at falling concentration curves and the curve for the free iodine in the plasma (were this the decisive factor) could then be drawn. If this were possible, any of the three methods would be equally satisfactory.

The aim of the foregoing discussion was to ascertain whether calculation of the clearance is possible after a single intravenous injection. It is seen that considerable sources of error arise but it is nevertheless evident that the more impaired the function, the smaller are the errors. If, therefore, using this method, differences are found between individuals or between groups, these differences are in reality greater than those obtained after comparison with a similar investigation of a normal material, for which the systematic errors are greater and the figures proportionately lower.

## CHAPTER III

### SURVEY OF THE CASES INVESTIGATED

The material consisted of the following groups: normal cases, acute and chronic nephritis, essential hypertension and a group with *one* functioning kidney. The groups were defined as follows:

**Normal Cases.** Individuals were considered as normal when there were no known symptoms of renal disease in their case-history and ordinary physical examination revealed no sign of disease. The principal part of this material consisted of healthy medical students, nurses and other female hospital personnel. Only a few were hospitalized patients with no renal disease (e.g. suffering from eczema, lumbago) all out of bed and afebrile. There were 56 individuals, 36 men and 20 women aged between 15 and 47 years, the majority being in the age-group 20-30 years (v. Table XI).

**Acute Nephritis.** This group comprised patients who, most frequently following angina or some other infection of the upper respiratory tract, had symptoms of acute renal disease with proteinuria, haematuria and in many cases raised blood-pressure and or oedema and increased non-protein nitrogen. In the case of one man, considerable sulphamide therapy had been given before his admission to hospital and renal injury owing to this medication could not therefore be eliminated. In two female patients, both over 55 years of age, there was reason to believe that they had

Table XI

Normal and pathological cases distributed according to age. Medians and quartiles are also given.  
 Figures in brackets indicate the number of deceased cases.

Age	Normal		Acute nephritis		Chronic nephritis		Hypertension		One functioning kidney	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
≤ 20 years.....	3	4	9	11	0	3(1)	1	0	0	1
21 — 30 " .....	26	11	20	8	7(2)	6(2)	0	0	4	1
31 — 40 " .....	6	3	10	9	4(1)	2	3(1)	5	3	2
41 — 50 " .....	1	2	9	3	4(2)	3(1)	3	7(1)	1	3
51 — 60 " .....	0	0	2	5	5(3)	3(2)	8(4)	14(2)	1	2
61 — 70 " .....	0	0	1	2	4(1)	4(2)	4	11(3)	0	0
> 71 " .....	0	0	0	0	1	2	1(1)	5(2)	0	1
Total	36	20	51	38	25(9)	23(8)	20(6)	42(8)	9	10
1 st quartile .....	21.9		21.1		26.9		47.0		28.0	
Median .....	25.7		29.3		42.9		55.9		38.0	
3 rd quartile .....	29.5		41.7		58.8		64.7		50.0	

both had elevated blood pressure several years before the onset of acute nephritis. Since, however, this disease was severe and acute they were included in the material, as was another woman, aged 52, where in addition to nephritis there was unilateral pyonephrosis with impaired function of the kidney involved, determined by urography.

The total number of patients in this group was 89 (51 men, 38 women). Ten patients, two men and eight women, were hospitalized more than 90 days after the onset of the disease and could therefore possibly be considered as sub-acute cases. Since, however, it is not possible to draw any sharp line between acute and sub-acute cases, their classification being more a matter of taste, they were also included. The majority of the other patients were hospitalized within 30 days after the onset of the disease as is seen in the following survey:

Admitted after:	Men	Women
5-10 days	15	7
11-15 »	10	5
16-20 »	6	8
21-25 »	5	2
26-30 »	7	4
31-35 »	3	1
36-40 »	1	1
41-90 »	2	2
>91 »	2	8

The majority of the patients were under 40 years of age, the youngest 13 years and the oldest 64 years old (v. Table XI). All the patients were treated in the Medical Department of the University Hospital in Upsala, with the exception of one man who was treated at a military hospital in the town under the supervision of the writer. Several of them were cared for in their homes before their stay in hospital and a few others were at other hospitals for a short period.

**Chronic Nephritis.** It is sometimes difficult to draw a dividing line between acute and chronic nephritis. For the purpose of the present investigation, patients who had suffered from nephritis for a considerable time (at least one year with the usual symptoms of this disease were considered as chronic cases. Retinal changes and cardiac hypertrophy can possibly be regarded as clinical signs of chronic nephritis. In some cases it was difficult to differentiate clinically between chronic nephritis and primary contracted kidney when no signs of an earlier nephritis or increased blood pressure were found or were noted in the case-history. Such patients that had pronounced urinary changes and in which the renal symptoms predominated were therefore included in this group.

This group consisted of 18 patients, 25 men and 23 women. In 21 cases there was definite proof that the renal disease had been present for more than 1 years and in a further 16 cases between 1 and 1 years. In 11 cases the symptoms had been present for less than one year but all the clinical symptoms of chronic renal disease were apparent. Seventeen (9 men and 8 women) of the 18 patients died later of uraemia. Post-mortem examination was performed on 10 of these and in every case the morbid-anatomical diagnosis was *nephrocirrhosis glomerulonephritica*. For the age-distribution see Table XI.

**Essential Hypertension.** This group consisted of patients without earlier known renal disease but whose blood pressure had either been raised for several years or who had suffered from subjective symptoms in the form of tachycardia, angina pectoris, headache, vertigo, or cerebral crises. Changes in the urine were less predominant and usually disappeared after a short stay in bed, thus indicating that they were caused more by cardiac than by renal factors.

The entire group consisted of 62 cases, 20 men and 42 women. There was a considerable predominance of women in this material. It must be emphasized, however, that the cases were not selected from the wards.

In 20 of the cases it was established that the disease had had a duration of more than four years, in 23 cases between one and four years. In the remaining 19 cases the rise in blood pressure had been revealed accidentally in connexion with acute illness.

Patients were classified as hypertensive when the systolic blood pressure exceeded 160 mm Hg and/or the diastolic pressure exceeded 90 mm Hg.

Fourteen of the 62 patients died during or a short time after hospitalization. Post-mortem examination was performed in 12 cases and in every case the clinical diagnosis was confirmed. For the age-distribution see Table XI.

**One Functioning Kidney.** Nineteen patients of this kind (9 men and 10 women) were investigated. Of these, 14 had been nephrectomized before the investigation: in 2 cases 8 and 14 days respectively before, 2 within 2 years, one within 3 years and another within 4 years before the investigation and the remaining 8 between 5½ and 23 years previously. Five patients had not been operated on, but X-ray examination revealed that only one kidney was functioning (determined by urography). The majority of patients in this group were under the age of 50 years (v. Table XI).

## CHAPTER IV.

### NORMAL INDIVIDUALS.

As mentioned previously, the normal material consisted of 56 individuals. Reference is made to Table XI (p. 95) for the distribution according to age and sex. Since all the determinations were not made in each case, the material was not sufficiently large to allow statistical calculations throughout for men and women separately. This was, however, possible for several tests and it was then found that no difference could be demonstrated between the sexes as regards inulin clearance, diodrast clearance, systolic and diastolic blood pressure and the filtration fraction (F.F.). A difference in the haematocrit figures was nevertheless found, a fact earlier known and obviously connected with the long-established fact that women have a smaller number of red blood corpuscles than men. Since the effective renal blood flow calculated is a function both of diodrast clearance and of the haematocrit (v. p. 42) the difference in this figure between the sexes is thus statistically significant.

In the case of creatinine clearance, urea clearance and the concentration of urea nitrogen, the determinations in one or the other sex were too few to permit a calculation of the standard deviation for each sex. The writer therefore proceeded as follows. When the material was too small for the calculation of the standard error for one sex only, the total material of both sexes was used to calculate the standard deviation. The respective standard errors were then calculated from this standard deviation, using the number of individuals in each sex. The standard error then certainly



becomes too large: in other words, the maximal standard error is obtained. As regards the calculations of the forementioned tests, i.e. creatinine and urea clearance, there is no reason to assume *a priori* that there should be any difference between the sexes since there was no statistically significant difference in this material between the sexes in the case of inulin or diodrast clearance. Moreover, the mechanism for the excretion of these various substances is partly the same and partly takes place by means of factors that are, in all probability not sex-linked, i.e. tubular reabsorption, secretion or rediffusion. The same is probably true of the urea nitrogen concentration in the plasma. All the normal values calculated for men and women separately, or for both sexes together if no statistically significant difference between them could be demonstrated, are found in Table XII.

The normal value for inulin clearance,  $124 \pm 2.2$  for men, is in good agreement with the figures published elsewhere (v. survey, p. 38) as is that for women,  $118.8 \pm 2.9$ . There was no statistically significant difference between the sexes in the writer's material, although the figure for women was lower. There was previously only one series in which such a calculation could be made, i.e. that published by Smith (1943). He found the mean figure for men to be  $131 \pm 2.63$  and for women  $117 \pm 3.40$ . The difference was calculated by the present writer as  $14 \pm 4.30$  and is therefore statistically significant. The question of the normal variability will be discussed later.

The present writer found the following normal values for diodrast clearance:  $448.9 \pm 7.0$  for men and  $436.4 \pm 10.0$  for women, thus no statistical difference between the sexes. There is, nevertheless, a numerical difference (-2.8 per cent) which, although it is far from statistically significant, is in the same direction but of a smaller order of magnitude than that found by Smith (1943), (-12.3 per cent). It is possible that if the present writer's material had been larger a greater difference would also have been found. Hilden (1946) likewise did not find any difference between the sexes in this respect, but his material was smaller than the present

Table XII. Mean (M), standard error of the mean ( $\epsilon$ ) and standard deviation ( $\sigma$ ) in different tests for adult normal men and women.

Determination <sup>1</sup>	Men			Women			Difference between men and women		Both sexes		
	Num-ber	M $\pm$ $\epsilon$ (M)	$\sigma$	Num-ber	M $\pm$ $\epsilon$ (M)	$\sigma$			Num-ber	M $\pm$ $\epsilon$ (M)	$\sigma$
Inulin clearance .....	36	124.1 $\pm$ 2.2	13.3	20	118.8 $\pm$ 2.9	12.8	5.3 $\pm$ 3.6		56	122.2 $\pm$ 1.8	13.2
Diodrast clearance .....	33	448.9 $\pm$ 7.0	40.1	20	436.4 $\pm$ 10.0	44.9	12.5 $\pm$ 12.2		53	444.2 $\pm$ 5.7	41.6
Filtration fraction .....	33	0.2764 $\pm$ 0.0050	0.0238	20	0.2735 $\pm$ 0.0073	0.0328	0.0029 $\pm$ 0.0088		53	0.2753 $\pm$ 0.0041	0.0208
Creatinine clearance ...	6	117.0	—	15	150.4 $\pm$ 5.1	19.9	—		21	140.9 $\pm$ 5.2	23.8
Urea clearance .....	15	82.4 $\pm$ 5.2	20.2	6	75.2	—	—		21	80.3 $\pm$ 3.9	17.9
Effect. renal blood flow	33	852.9 $\pm$ 11.1	63.9	20	762.9 $\pm$ 14.8	66.4	90.0 $\pm$ 18.5		—	—	—
Hematocrit .....	36	47.38 $\pm$ 0.49	2.96	20	42.78 $\pm$ 0.64	2.84	4.60 $\pm$ 0.81		—	—	—
Blood pressure { systolic	36	130.4 $\pm$ 1.3	7.6	20	130.5 $\pm$ 1.7	7.6	0.1 $\pm$ 2.1		56	130.4 $\pm$ 1.0	7.5
diastolic	36	75.83 $\pm$ 0.70	4.23	20	77.0 $\pm$ 1.22	5.48	1.17 $\pm$ 1.41		56	76.25 $\pm$ 0.62	4.65
Urea nitrogen, mg % ...	17	17.21 $\pm$ 0.97	3.99	7	18.79	—	—		24	17.67 $\pm$ 0.75	3.70

<sup>1</sup> Inulin, diodrast and creatinine clearances and the effective renal blood flow are in the tables calculated in ml/min. Urea clearance expressed in per cent of a normal value (100 %). All clearance figures refer to 1.73 sq. m. Blood pressure in mm Hg.

writer's. It can only be said that if a difference between the two sexes does exist, it cannot be very great. As regards the absolute values, those given here are lower than those of the majority of workers but this depends on the technique used for injection of the iodine contrast and possibly to some extent on the preparation used. Those workers who used a single intravenous dose (White et al., Hilden) as did the present writer, give a value around 410. This is approximately of the same order of magnitude as the figures in the present investigation. After subcutaneous injection, and particularly after continuous intravenous infusion, the figures are higher but the variability is also greater. It is also possible, as mentioned previously, that the nature of the iodine preparation could contribute to figures that are lower than those of the majority of workers. In the present investigation hippuran was used as a rule, and according to Elsom et al. (1934, 1936, 1937) and Smith et al. (1938) hippuric acid iodine gives a lower clearance than pyridine-iodine compounds (per-abrodil, diodrast). The difference is nevertheless extremely small, and the writer — who in many cases made clearance tests with both preparations on the same subject but on different days — was unable to find any perceptible difference between them.

Creatinine clearance was only investigated in 6 men and the mean value was 117. This is low in comparison with the value for women,  $150.4 \pm 5.1$  on whom the majority of tests were made, but can possibly be due to the fact that the tests on men were made with a different creatinine preparation. Moreover, the material was too small for an exact calculation. There is no reason to expect lower values for men than for women; on the contrary, should such a difference exist, it would probably be in the opposite direction. As emphasized earlier, Smith found higher inulin and diodrast values for men, and the difference in body size in the two sexes indicates that this should also be the case as regards creatinine. The present writer was unable to find any material in the literature in which the creatinine clearance was differentiated for the two sexes, and in the

present investigation a calculation was therefore made for both sexes together in order to facilitate a comparison with the results of other workers. The mean value for 21 healthy individuals was  $110.9 \pm 5.2$ , which is in good agreement with that found by other writers (Lassen 1932; Hayman, Halsted and Seyler 1933; Ekehorn 1944, v. p. 38).

The variability (i.e. standard deviation) of the creatinine clearance is 23.8 or, expressed as a percentage of the mean, 16.9 per cent. The corresponding figure for inulin clearance in the present material is 13.2 and the variation coefficient 10.8 per cent. The difference between these two figures is almost statistically significant, i.e.  $10.6 \pm 3.9$ . A minor part of the difference can be caused by the fact mentioned in the foregoing, i.e. the few determinations on men were made with a preparation which possibly gives values on a lower level. If these figures are compared with others published, it is seen that the majority of writers found a similar difference. Only two investigations (Friedman; Josephson and Lindahl) have given approximately the same variability. Since no statement concerning the age-distribution is given in their series, it is not possible to decide whether the results can depend, for example, on differences in ages in the materials. It should therefore be emphasized that the present determinations were carried out on individuals mainly between the ages of 20 and 30 years (cf. Table XI).

It is of secondary interest to demonstrate that the variability is less in one individual than between several individuals. This is not evident from the present investigation but can be shown from material in the literature. As mentioned previously, Ekehorn collected a normal material in which a number of determinations carried out on the same individual were included. The individual variability can be calculated from these figures. In Ekehorn's series 35 determinations were made on one individual and 44 on another. If these figures only are used, an idea of the individual variability is obtained (v. Table XIV).

It is seen that the standard deviation is 20.5 for the first-mentioned individual and only 14 for the last-mentioned.

Table XIII

Mean (M), standard deviation ( $\sigma$ ) and coefficient of variation (v) in different tests for adult normal persons of both sexes.

Determination	Number	M	$\sigma$	v
Inulin clearance .....	56	122.2	13.2	10.8
Diodrast clearance .....	53	444.2	41.6	9.4
Filtration fraction .....	53	0.2753	0.0298	10.8
Creatinine clearance .....	21	140.9	23.8	16.9
Urea clearance .....	21	80.3	17.9	22.3
Effect. renal blood flow ....	53	818.9	77.1	9.4
Hæmatocrit .....	56	45.73	3.61	7.9
Blood pressure { systolic ...	56	130.4	7.5	5.8
	56	76.25	4.65	6.1
Urea nitrogen, mg % .....	24	17.67	3.70	20.9

Table XIV

Mean (M), standard error of the mean ( $\epsilon$ ) and standard deviation ( $\sigma$ ) for a single individual, determined by repeated tests, and for different individuals.

Investigator	Number	M $\pm$ $\epsilon$ (M)	$\sigma$
Determinations on the same individual (Rehberg) .....	35	125.6 $\pm$ 3.5	20.5
Determinations on the same individual (Poulsson) .....	44	158.3 $\pm$ 2.1	14.0
Determinations on different individuals (Gårdstam) .....	20	144.6 $\pm$ 7.9	35.3
Determinations on different individuals (Cambier) .....	43	136.2 $\pm$ 5.1	33.2

The standard deviations between various individuals, obtained from the same source, are also shown in the table. As can be expected, the latter figures are considerably higher, i.e. 33 and 35. The impression received from these figures is that the individual variation is approximately half as large as between different individuals. This, of course, opens up a possibility of obtaining different means for normal individuals by selecting different materials.

As in the case of creatinine clearance, it was necessary to calculate the urea clearance for both sexes together, since the determinations made on women were too few to permit a statistical comparison between the sexes. The value obtained is  $80.3 \pm 3.9$ , which contrary to the other clearance values, is expressed in the usual way as a percentage of a standardized figure of 75 and 51 ml respectively per minute. This is lower than that usually given in the literature (around 100 per cent). The reason is probably that in making determinations of the urea nitrogen concentration in the urine with the method used in the present investigation, figures proportionately too low are obtained, whereas determinations in the plasma give higher figures (v. Chapter II). For the purpose of the present investigation, it is probably of little importance whether the normal figure is 80 or 100 per cent, but this fact must naturally be borne in mind in the discussion which follows.

Since the value for diodrast clearance is lower here than that generally stated in the literature, the value for the effective renal blood flow is obviously also comparatively lower.

The haematocrit figures found in the present investigation are in good agreement with those given by Whitby and Britton (1947) but higher than those published by Eng-hoff (1937) possibly owing to differences in the method used.

The figures for blood pressure and the urea nitrogen concentration in the plasma are also normal according to current interpretations.

The filtration fraction (F.F.) — or the ratio of the inulin to the diodrast clearance — is also obviously higher than that given by other workers, a fact likewise to be attributed to the lower values obtained by the present writer for the diodrast clearance.

On a comparison between Table XII, in which the present writer's normal values for the inulin, creatinine and diodrast clearances are given, and Tables I and II which give those of other workers, it is seen that the standard deviation for all these three tests is lower in the present material than

in those published earlier. The variability in series such as these can depend on several factors, particularly on differences in the materials. When, as here, healthy individuals are involved — it must be admitted that definite criteria for their classification as such are present — differences in the material can depend chiefly on the varying ages of the individuals investigated. In addition, the size of the standard deviation can depend on different methods of determination or varying accuracy in carrying out the method. Since it was usually impossible for the present writer to obtain exact information on the forementioned points as regards earlier publications, an explanation of the lower standard deviations in the present material must be confined to the following.

1. The material comprised a relatively limited age-group with the majority of cases between the age of 20 and 30 years. Moreover, the subjects were nearly all engaged in medical work and thus fully conscious of the importance of following minutely all instructions given.

2. All the determinations were performed simultaneously on the same blood and urine sample.

3. All the urine samples in this normal series were voided spontaneously and the volume of urine was kept so high that there was every occasion to assume that the bladder was properly emptied. It is true that a better guarantee for complete emptying of the bladder is considered to exist if catheterization and washing out of the bladder are used. This is not, however, the experience of the present writer on the basis of all the cases of senile or severely ill patients where it was necessary to use this method, as considerably larger variations then occurred in the clearance tests. Whether this latter fact was due to faulty technique or to a secondary effect of the intervention on the renal function must remain an open question.

4. All the analyses were performed in exactly the same way and by the same laboratory worker. Moreover, all the figures were based on the mean for three clearance periods of 20 minutes each.

5. In all clearance determinations the standard deviation

is obviously lower the lower the figure obtained. As far as diodrast clearance is concerned, this is evident from Table II, and since the present writer's figures for this determination are lower than those of the majority of other workers, the explanation of the lower variability can, at least in part, be attributed to this factor.

As mentioned in the foregoing, it was necessary for the writer to establish his own normal values for the tests used. This was particularly desirable to allow accurate comparison of the normal values with those obtained for the pathological cases, but also since the normal values in the literature are usually based on a small number of determinations and can thus scarcely be considered to provide adequate information.

Normal values are required both in order to judge the individual cases of illness and to compare these cases as a group. The question is then whether the pathological cases differ on the whole from the normal ones, and then the means are mainly used. When individual cases are to be judged, the mean figure for normal individuals no longer suffices; it is also necessary to know the extent of the variations in the latter. In Table XV the borderline figures for the normal variations are therefore given first, calculated in the usual way with the help of three times the standard deviation. A single figure outside this limit is a definite deviation from the normal occurrence. A figure within the established normal limit but comparatively near it can be normal, although this is seldom the case. When diseased persons are involved, it is not possible to wait in each case until a definite diagnosis is obtained. A zone between 2 and  $2\frac{1}{2}$  from the mean figure is therefore indicated within which normal figures are very rare. If such a figure is obtained for a patient, it can be said that it is possible, although not probable, that he is normal. The final diagnosis in such cases must also depend on whether other pathological symptoms are present. As is usually the case when a diagnosis is made, this must be based on a number of probabilities and the final judgement is therefore not absolutely definite but has only a certain probability.



Table XV

Mean ( $M$ ), standard error of the mean ( $\varepsilon$ ) and standard deviation ( $\sigma$ ) for normal individuals in tests which show no sex differences. The limits for the range of normal variation according to 2  $\sigma$ , 2.5  $\sigma$  and 3  $\sigma$  are also given.

Determination	Num- ber	$M \pm \varepsilon(M)$	$\sigma$	Limits for the range of normal variation		
				$M \pm 2\sigma$	$M \pm 2.5\sigma$	$M \pm 3\sigma$
Inulin clearance .....	56	$122.2 \pm 1.8$	13.2	96 — 149	89 — 155	83 — 162
Diodrast clearance .....	53	$444.2 \pm 5.7$	41.6	361 — 527	340 — 548	319.4 — 569
Filtration fraction .....	53	$0.2753 \pm 0.0041$	0.0298	0.216 — 0.335	0.201 — 0.350	0.186 — 0.365
Creatinine clearance .....	21	$140.9 \pm 5.2$	23.8	93 — 189	81 — 200	69 — 212
Urea clearance .....	21	$80.3 \pm 3.9$	17.9	44 — 116	35 — 125	26 — 134
Urea nitrogen, mg % .. ...	24	$17.67 \pm 0.75$	3.70	10.3 — 25.1	8.5 — 26.9	6.7 — 28.7

## CHAPTER V.

### GLOMERULONEPHRITIS

Interest in the study of renal function in glomerulonephritis has naturally existed ever since renal functional tests came into being. With the earlier methods used it was, nevertheless, extremely difficult — if not impossible — to make an exact diagnosis of the actual condition of the kidney. The following statement made by Lunds gaard (1930) is typical of this outlook: «the conditions of renal function are thus not suitable for deciding whether an acute nephritis has regressed or become chronic». A functional diagnosis would, however, be invaluable for assessing the degree of renal injury in acute nephritis, whether this has improved or not, etc. This is all the more desirable since many cases of acute nephritis appear to have improved, in so far as the urine is normal and also the blood pressure but nevertheless, after a varying period of latency, symptoms of chronic nephritis appear. Moreover, it is often difficult to decide whether nephritis is chronic or not, e.g. when only proteinuria and a slight sediment are present but the «peripheral» signs (rise in the blood pressure, cardiac hypertrophy or retinal changes) are absent. There are even examples in the literature of cases in which these «peripheral» signs were lacking during the whole period of the disease but the cause of death was nevertheless uraemia (Jores 1908, Foster 1922 and Bannick 1927). In the following, the literature in regard to acute as well as chronic nephritis will be shortly reviewed.

Holten (1931, 1933) studied the creatinine clearance and

the concentration test in acute nephritis in children and concluded that it is possible to obtain a localized diagnosis with the help of these two tests: decreased creatinine clearance indicates impaired glomerular filtration and decreased concentration capacity indicates tubular damage. He drew a number of curves to illustrate that creatinine clearance usually becomes normal only after proteinuria and positive sediment are no longer present and the concentration capacity has become normal. He nevertheless also reported several cases in which the concentration capacity had been normal during the whole illness despite a considerable decrease in creatinine clearance. The latter condition, i.e. normal concentration during the whole course of acute nephritis, was also pointed out earlier by Volhard (1918). Hollen was, however, of the opinion that such a localized diagnosis was impossible in chronic nephritis since the whole nephron is then injured.

Raaschou (1943) also discussed the question of dissociation between glomerular and tubular function in renal disease and based his arguments on Fishberg's (1939) classification of renal insufficiency into three types: 1) glomerular, 2) tubular and 3) combined glomerular and tubular type. According to Fishberg, glomerular insufficiency is characterized by reduced filtration with unimpaired tubular function, the symptoms being oliguria, high specific gravity of the urine, reduced glomerular clearance and normal concentration capacity; typical conditions are severe peripheral circulatory and cardiac insufficiency. Tubular insufficiency is manifested by reduced tubular reabsorption, excretion and synthesis but unimpaired filtration, the clinical symptoms being polyuria, reduced specific gravity and normal glomerular clearance, as for example, in cases of diabetes insipidus. In Bright's disease, and other bilateral renal diseases the combined type is found, when at times the picture is dominated by glomerular insufficiency and at times by tubular. Raaschou quoted a case of hyperparathyroidism with calcifications in the renal medulla of which an account was published by Hollen (1936) and two cases of chronic

nephritis reported by Holten and Rehberg (1931) in which the creatinine clearance was normal but the concentration capacity reduced. He added a number of his own cases of acute pyelitis and chronic pyelonephritis in which the concentration capacity was also reduced but glomerular function (determined by urea clearance!) was normal, and considered that a combination of these two tests permits a localized diagnosis.

Cullen, Nelson and Holmes (1935) determined the urea clearance in sixteen children during the acute stage of nephritis and during convalescence. They found that, with the exception of one case, the clearance figures were low during the initial stage but that after one month the majority showed normal figures and that at the same time the urea in the blood had decreased and other symptoms of acute nephritis had regressed. In a group of 78 children with a history of acute haematuric nephritis they found that the urea clearance was  $109.1 \pm 1.5$  per cent compared with  $107.3 \pm 1.4$  per cent in a control group of healthy children in the same age-group. Some of the children in the former group had relatively low urea clearance or other symptoms of kidney damage (usually proteinuria) but during a long observation time the clearance showed rising figures and the proteinuria disappeared or decreased.

In early stages of acute nephritis Winkler and Parra (1937) were able in a few cases to find disproportionately high creatinine clearance, but as a rule the clearances of creatinine, sucrose and urea were uniformly reduced in subjects with renal disease. Both the absolute and the relative variability of the clearances were reduced and the ratio between these clearances approached the figure 1. The forementioned writers interpreted this as a possible decrease in tubular secretion of creatinine and reabsorption of sucrose with progressive renal damage. Were this the case, the rediffusion of urea should be the same in patients with damaged kidneys as in healthy individuals. Chasis and Smith (1938) also considered that they had demonstrated this to be true but, as mentioned in the foregoing (v. p. 26)

Ekehorn (1946) gives some reasons why the more severe the renal damage the more urea is reabsorbed. It can be mentioned in this connexion that it was not possible to demonstrate any such rediffusion or reabsorption of inulin in patients with chronic nephritis (Miller, Alving and Rubin 1940) and therefore, *inter alia*, the inulin clearance is probably an expression of the actual volume of the filtrate even in impaired kidneys.

The clearances of inulin and phenol red were investigated by Goldring and Smith (1937-1938) in 21 patients suffering from acute or chronic nephritis. They found that, as a rule, the quotient of the ratio of phenol red clearance to inulin clearance remained within normal limits, indicating that glomerular and tubular damage progress parallel to each other. In more advanced cases, however, this quotient decreased and this was interpreted as an inability of the tubules to excrete the dye. In a few cases, which had been observed earlier during the acute stage, this quotient was so much in excess of the normal that a dissociation of glomerular and tubular function could be suspected. In one case, for example, the inulin clearance was 72 per cent of the normal figure, whereas the phenol red clearance was 156 per cent, but after some months approached a normal figure. The formentioned writers considered that a possible explanation of this phenomenon could be a dilatation of the efferent arterioles of the glomeruli, since this not only tends to increase the phenol red clearance through increased blood flow but also to decrease filtration through lowered filtration pressure.

In 1941 Steinitz published a paper on renal blood flow in *inter alia* two patients with acute nephritis on whom determinations of inulin and diodrast clearance were made. Both patients were severely ill with considerably raised blood urea (one later died of uraemia) and the clearances were low but proportionately lower for inulin than for diodrast, thus giving a low filtration fraction. In a discussion of these cases the writer pointed out that it is naturally impossible to draw any conclusions from them regarding renal blood flow in acute nephritis but he referred to Sarre's

(1939) investigation of the renal blood flow in the rabbit with experimental nephritis (produced by Masugi's method) in which it was shown that the flow, determined with Rein's thermostromuhr, was satisfactory at every stage of the acute nephritis. Earle Taggart and Shannon (1941) obtained similar results in a study of 22 patients in various stages of glomerulonephritis in which they made determinations of the glomerular filtration (inulin), renal plasma flow (diodrast) and the maximal tubular excretion of diodrast ( $T_{mD}$ ). They summarized their conclusions as follows: 1) All these three functions are decreased when the disease progresses, and marked changes appear in their normal ratios. Filtration is the most sensitive indicator of renal impairment at an early stage of the disease and is accompanied by a low filtration fraction and a low ratio of the inulin clearance to  $T_{mD}$ , as well as a decrease in inulin clearance. 2) Acute nephritis and exacerbation of chronic nephritis are usually associated with a decrease in the inulin clearance,  $T_{mD}$ , the filtration fraction and the ratio of inulin clearance to  $T_{mD}$ . All or some of these figures may rise when improvement occurs, but a high ratio of the diodrast clearance to  $T_{mD}$  may be explained by transient hyperaemia. No definite correlation was found between the specific changes in renal function or between their respective ratios and the prognosis of the disease. 3) When chronic nephritis progresses, tubular function is relatively more impaired than glomerular filtration and the diodrast clearance therefore shows a proportionately larger decrease than that of inulin, and the ratio of inulin clearance to  $T_{mD}$  rises.

Hilden (1913, 1941, 1946), apparently altogether unaware of the forementioned investigations, came to practically the same conclusions in an investigation of nephritic patients by means of urea and diodrast clearances. In mild and moderately severe cases of acute nephritis he found a decrease in urea clearance whereas that of diodrast was often normal or showed a proportionately less pronounced decrease. He found the same conditions in cases of severe nephritis. As a result, the quotient of urea clearance to dio-

drast clearance was decreased in the same way that earlier investigators found to be the case for the filtration fraction. Hilden concluded that glomerular function is more affected in acute nephritis than tubular function and gives three possible reasons:

1. The pathological changes are localized to the glomeruli.
2. Dilatation takes place in the renal efferent arterioles.
3. The extra-glomerular blood flow to the tubules is increased.

He did not, however, state which of these three possibilities he considered to be the most probable, but this question was discussed thoroughly by Black, Platt, Rowlands and Varley (1948) who investigated the inulin and diodrast clearances in three patients with acute nephritis and came to the same conclusions as Hilden. They also put forward three possibilities to explain the proportionately greater damage to filtration:

1. Decreased permeability of the glomerular membrane.
2. Some of the blood is «shunted» past the glomeruli.
3. A change occurs in the tonus of the afferent or efferent arterioles.

They rejected the first two reasons and considered the third to be the most probable. They made a calculation of the tonus in the afferent and efferent arterioles on the basis of Lampion's (1941) formulae and concluded that the afferent resistance is considerably increased during the acute hypertensive phase when the efferent resistance remains normal. They consider the low filtration rate to be due chiefly to a fall in filtration pressure.

Hilden also studied clearance conditions in chronic nephritis. He summarized his conclusions, based on 26 cases, as follows:

In the latent stage of chronic nephritis, normal or slightly decreased clearance figures can be found, the decrease being equally pronounced in the case of urea and diodrast clearance.

In the chronic active stage a difference is found, depending on the type of the disease: the hypertensive type exhibits

a larger decrease in the diodrast than in urea clearance, whereas the conditions are reversed in the nephrotic type.

In the final stage there is a considerable decrease in both clearances, but this is most pronounced in the case of diodrast.

## Acute Nephritis

As previously mentioned, the diagnosis of acute nephritis has been based on the presence of proteinuria and haematuria, often following an infection of the upper respiratory tract. In addition, many cases have shown an elevated blood pressure and/or symptoms associated with a disturbance of the renal function.

The present writer was able to make an investigation on 89 patients (51 men and 38 women) in various stages of acute nephritis. The age-distribution of the material is seen in Table XI and on page 94 further information is given about the cases. It is seen from the table that the majority of the patients belong to the lower age-groups, 54 per cent being under the age of 30 years and 75 per cent under the age of 40. In this respect the material is in good agreement with large series published earlier. Thus, for example, Rudebeck (1946) had 53.8 per cent under the age of 30 and 74 per cent under the age of 40 years in his material of 318 patients. The present material can thus — at any rate from this aspect — be considered as representative of an unselected group of nephritics in a medical ward, all over the age of 12 years. The age-distribution is further demonstrated by the fact that the median lies at 29 years, the lower quartile at 21 years and the upper quartile at 42 years. In the present material, as in papers published earlier, the distribution according to sex shows a preponderance of men, the ratio of men to women being approximately 3:2, this being more marked in the lower age-groups (cf. Gachel 1941, Rudebeck 1946). In another respect, i.e. the mortality, the present material differs, however, from those earlier published. In Rudebeck's material, for example, the mortality rate was



6.6 per cent, whereas there were no deaths in the present writer's material. This difference can be due to random variation but is probably dependent on the fact that in the present material none of the patients who were severely ill during hospitalization were subjected to these tests. The material was thus a selected one of not extremely severe cases, and this can explain the lack of deaths.

Data concerning the duration of the disease before admission to hospital are found in the table on page 96. It is seen that nine patients were hospitalized more than three months after the onset of the disease (the remainder being admitted earlier) and they should therefore perhaps have been classified as subacute. Nevertheless, a number of those patients who were admitted at an earlier stage of the disease still showed symptoms of nephritis after three months. This could not have been anticipated when the functional tests were made and these patients were therefore included among those with acute nephritis although — with regard to the duration of the disease — they should possibly have been considered as subacute. It is, however, difficult to make this distinction and it is also of less importance in the present paper in which *inter alia* the following factors were investigated: a) the relation of the current symptoms to the actual renal function and b) the relation of the latter to the time for recovery. The borderline was in that respect fixed at three months. It can be pointed out that in principle there is naturally no sharply-defined borderline either between acute and chronic cases or between chronic and imperfectly healed cases. All borderlines must be more or less subjective, even if there is no doubt concerning the majority of cases.

In order to obtain a conception about the state of renal function in patients with acute nephritis, these can be compared with normal individuals, both as a group according to the respective mean figures and individually according to the known upper limit for the normal individuals. Furthermore, the cases can be analysed in regard to the correlation between the different tests as well as in regard to the correlation between the different symptoms and the tests.

Table XVI

Mean (M), standard error of the mean ( $\pm$ ), and standard deviation ( $\sigma$ )  
in different tests for men and women with acute nephritis.

Determination	Men			Women			Both sexes		
	Num- ber	M $\pm$ $\pm$ (M)	$\sigma$	Num- ber	M $\pm$ $\pm$ (M)	$\sigma$	Num- ber	M $\pm$ $\pm$ (M)	$\sigma$
Inulin clearance .....	51	92.0 $\pm$ 4.9	34.7	38	80.4 $\pm$ 5.3	32.6	89	87.1 $\pm$ 3.6	34.1
Diodrast clearance .....	51	389.7 $\pm$ 15.2	108.2	38	322.6 $\pm$ 18.0	111.2	89	361.0 $\pm$ 12.1	113.8
Filtration fraction .....	51	0.234 $\pm$ 0.009	0.064	38	0.252 $\pm$ 0.012	0.072	89	0.242 $\pm$ 0.007	0.067
Creatinine clearance .....	17	92.9 $\pm$ 8.6	35.3	19	88.3 $\pm$ 9.4	41.1	36	90.5 $\pm$ 6.3	38.0
Urea clearance .....	25	71.7 $\pm$ 6.6	32.8	19	52.3 $\pm$ 4.1	18.1	44	63.3 $\pm$ 4.3	28.8
Effect. renal blood flow ...	51	670.5 $\pm$ 27.9	199.2	38	537.8 $\pm$ 31.1	191.9	89	613.8 $\pm$ 21.8	205.8
Hæmatoerit .....	51	41.02 $\pm$ 0.75	5.37	38	39.24 $\pm$ 0.88	5.41	89	40.26 $\pm$ 0.58	5.43
Proteinuria <sup>1</sup> .....	45	0.48 $\pm$ 0.16	1.07	37	0.53 $\pm$ 0.27	1.63	82	0.50 $\pm$ 0.15	1.33
Blood pressure { systolic ..	51	130.5 $\pm$ 1.7	12.0	38	139.9 $\pm$ 3.9	23.8	89	134.5 $\pm$ 2.0	18.4
diastolic ..	51	81.4 $\pm$ 1.5	10.4	38	87.2 $\pm$ 2.4	14.6	89	83.9 $\pm$ 1.3	12.6
Urea nitrogen, mg % .....	27	22.9 $\pm$ 2.3	12.1	22	19.3 $\pm$ 1.1	5.1	49	21.2 $\pm$ 1.4	9.7

<sup>1</sup> Expressed in ‰.

When the cases of acute nephritis are to be compared as a *group* with the normal material, the results of the investigation are seen in Table XVI.

For both sexes together there was a statistically significant difference for all the determinations with the exception of the systolic blood pressure and urea nitrogen. In both these tests the difference was nevertheless approximately twice the mean error (Table XVII).

Table XVII.

Differences and standard errors of the differences between means for normal cases and means for cases with acute nephritis in regard to different tests. A minus difference signifies that the normal figure is larger, a plus difference signifies the contrary.

Determination	Differences between normal cases and cases with acute nephritis		
	Men	Women	Both sexes
Inulin clearance . . . . .	-32.1 $\pm$ 5.4	-38.4 $\pm$ 6.0	-35.1 $\pm$ 4.0
Diodrast clearance . . . .	-59.2 $\pm$ 16.7	-113.8 $\pm$ 20.6	-83.2 $\pm$ 13.4
Filtration fraction . . . .	-0.043 $\pm$ 0.010	-0.021 $\pm$ 0.014	-0.034 $\pm$ 0.008
Creatinine clearance . . . .	-24.1 $\pm$ 13.0	-62.1 $\pm$ 10.7	-50.4 $\pm$ 8.2
Urea clearance . . . . .	-10.7 $\pm$ 8.4	-22.9 $\pm$ 8.4	-17.0 $\pm$ 5.8
Effect. renal blood flow . .	-182.4 $\pm$ 30.0	-225.1 $\pm$ 34.4	-205.1 $\pm$ 24.2
Hematocrit . . . . .	-6.36 $\pm$ 0.90	-3.54 $\pm$ 1.09	-5.47 $\pm$ 0.75
Blood pressure	(systolic	+ 9.4 $\pm$ 4.3	+ 4.1 $\pm$ 2.2
	(diastolic	+ 10.2 $\pm$ 2.7	+ 7.6 $\pm$ 1.4
Urea nitrogen, mg % . . .	+ 5.7 $\pm$ 2.5	+ 0.5 $\pm$ 1.8	+ 3.5 $\pm$ 1.6

This was also the case for men and women separately, with the exception of the creatinine clearance for which there was no significant difference for the men. This was probably due to the fact that the creatinine clearance level is lower for men than for women in the writer's normal material. This was discussed earlier (v. Normal Individuals) and the explanation suggested was that the clearance was determined with another creatinine preparation. That this is the most probable reason is supported by the experience of the present writer, but not earlier mentioned, i.e. that creatinine preparations of different brands give very varying clearance

values. Thus, for example, one or two brands gave figures far below the inulin clearance determined simultaneously. This could also be a natural explanation of the different normal values given by different workers. It is obvious that this factor must be taken into consideration when normal values are calculated from the results of different writers.

As regards the urea clearance, there was no demonstrable difference compared with the normal material in the male group, but the difference was statistically probable for the women and for both sexes together. This could be caused by all the figures for the women being somewhat lower than for the men, the group of women thus representing somewhat more severe cases. This could also — at least to some extent — be explained by variations in the urea excretion with falling filtration rates (v. General Discussion). The difference may, nevertheless, be purely fortuitous.

It must be pointed out that the filtration fraction was lower in this group of patients than in the normal material. In other words, there was a smaller decrease in the diodrast than in the inulin clearance. The difference was statistically significant.

It is seen from Table XVI that the decrease in the filtration rate, determined with inulin or creatinine clearance, was approximately 30 per cent, whereas there was a decrease of only 19 per cent in the diodrast clearance. The mean figures for the urea nitrogen in the plasma were 20 per cent higher than in the normal material, but the increase in the diastolic blood pressure was only 10 per cent and in the systolic pressure 3.5 per cent, compared with the normal levels. All these calculations are in respect of the cases of acute nephritis as a group.

As regards the *individual* figures, a calculation can be made of how large a part of the material lies outside the limits  $2\sigma$  and  $3\sigma$  of the normal material. Such a calculation has been made in Table XVIII as regards those functional tests which for the normal material showed no statistically significant sex differences.

It is seen from this table that 43 per cent of all the cases

had a lowered filtration rate whereas 35 per cent showed a decreased diodrast clearance when the range of normal variation was fixed at  $3\sigma$ . At  $2\sigma$  the number of cases with decreased figures was obviously larger. The difference between those who showed decreased figures using these borderlines naturally varied owing to the small size of the material. Such variations as regards the differences should thus not be considered of importance. It can, nevertheless, be considered as established that the percentage of pathological cases is lower for the urea clearance and urea nitrogen.

Table XVIII

Number and percentage of individuals with acute nephritis falling outside the normal limits calculated according to  $2\sigma$  and  $3\sigma$  respectively.

Determination	No.	$2\sigma$	$3\sigma$
Inulin clearance	89	49 (55 %)	38 (43 %)
Diodrast clearance	89	36 (40 %)	31 (35 %)
Filtration fraction	89	34 (38 %)	22 (25 %)
Creatinine clearance	36	17 (47 %)	10 (28 %)
Urea clearance	44	12 (27 %)	3 (7 %)
Urea nitrogen, mg %	49	9 (18 %)	5 (10 %)

It is possible that in nephritis different partial functions of the kidney are more or less injured. It can be seen from the foregoing that in this material all measurable renal functions were affected. It is thus of interest to ascertain whether some particular aspect of renal function was more impaired than another and also to endeavour to elucidate whether the conditions differ in different patients, i.e. whether in one patient the injury to the glomerular apparatus is predominant or whether tubular function is more impaired.

Since certain of the tests used refer to the same part of the nephron, it can be expected that their results would be similar, i.e. if one test indicates a pathological disturbance, the other should also do so. Such a correlation can, however, also depend on the fact that the tests refer to different parts

of the nephron but that in acute nephritis these are always damaged simultaneously. A calculation intended to throw light on these questions is found in Table XIX, in which the observed and the expected figures for the borderlines  $2\sigma$  and  $3\sigma$  are given as before. The expected figures are obtained simply by multiplication of the percentage figures in Table XVIII. These figures are to be expected if the disturbances according to the forementioned borderline figures have an entirely random distribution. If, on the other hand, a correlation exists between the decrease in two tests, the figure for the observed value rises. The greater the difference between the expected and the observed figure, the greater the correlation between the decrease in the two tests. In other words, this method is a simple way of demonstrating the correlation between the tests.

Table XIX shows that a definite correlation can be demonstrated only between a decreased inulin clearance and a decreased filtration fraction and between a decrease in the urea clearance and an increase of the urea nitrogen. In addition there is a statistically probable correlation between the decrease in the inulin and the diodrast clearances, the inulin and creatinine clearances and the filtration fraction and the creatinine clearance. The material is unfortunately too small to permit any further conclusions but in all probability such a calculation on a larger material could give valuable information on how the damage caused by acute nephritis affects different parts of the nephron.

The closest correlation seems to exist between the inulin and creatinine clearances, although the material is small and the correlation cannot therefore be statistically significant here. Since both these tests are mainly an expression of the filtration capacity, their results could be expected to be similar.

A fairly marked increase in the observed figures for diodrast and creatinine is obviously expected since both these tests — although to a different extent — are conditioned by tubular function. It is, however, evident that the inulin and diodrast clearances are also correlated and this fact is prob-

Table XIX  
Expected and observed values (in per cent) of cases with acute nephritis  
outside certain limits for normal values (2 σ and 3 σ)  
in pairs of different tests<sup>1</sup>.

Determination		Number	Expected value, %		Observed value, %	
			2 σ	3 σ	2 σ	3 σ
Inulin clearance	— Diodrast clearance	89	22 ± 4.4	15 ± 3.8	34	29
Inulin clearance	— Filtration fraction ..	89	21 ± 4.3	11 ± 3.3	37	22
Inulin clearance	— Creatinine clearance	36	26 ± 7.3	12 ± 5.4	47	28
Inulin clearance	— Urea clearance ....	44	15 ± 5.4	3 ± 2.6	27	7
Inulin clearance	— Urea nitrogen, mg %	49	10 ± 4.3	4 ± 2.8	18	10
Diodrast clearance	— Filtration fraction ..	89	15 ± 3.8	9 ± 3.0	20	14
Diodrast clearance	— Creatinine clearance	36	19 ± 6.5	10 ± 5.0	31	25
Diodrast clearance	— Urea clearance ....	44	11 ± 4.7	2 ± 2.1	20	7
Diodrast clearance	— Urea nitrogen, mg %	49	7 ± 3.6	4 ± 2.8	14	8
Filtration fraction	— Creatinine clearance	36	18 ± 6.4	7 ± 4.3	36	17
Filtration fraction	— Urea clearance ....	44	10 ± 4.5	2 ± 2.1	25	2
Filtration fraction	— Urea nitrogen, mg %	49	7 ± 3.6	3 ± 2.4	14	6
Creatinine clearance	— Urea clearance ....	13	13 ± 9.3	2 ± 3.9	54	15
Creatinine clearance	— Urea nitrogen, mg %	15	8 ± 7.0	3 ± 4.4	20	13
Urea clearance	— Urea nitrogen, mg %	42	5 ± 3.4	1 ± 1.5	17	5

<sup>1</sup> The expected values are obtained simply as a product of the frequency of the respective tests (v. Table XVIII) and should apply if there was only a random combination.

ably chiefly an expression of impairment of both glomerular and tubular function. The fact that both are impaired in acute nephritis naturally decreases the possibility of drawing any particular conclusions regarding the special nature of the various tests.

To a certain extent the combination of urea clearance and urea nitrogen occupies a unique position. It is not a question, as in the previous cases, of a combination of two tests, but of a combination of the concentration of a certain substance in the blood with the clearance of the respective substance. It could be expected that when the clearance was

low the concentration in the blood would always be high. This is often but not, however, always the case. As mentioned previously, the correlation is significant.

Regarding the question whether glomerular function is more impaired in an individual case than the tubular function or *vice versa*, this is more difficult to decide since no specific tubular functional tests were performed. The diodrast clearance only was determined and this — as stated previously in Chapter I — is dependent both on the effective renal blood flow and on the secretory power of the tubules. In other words, a decreased diodrast clearance can either indicate that less blood flows through the kidney or that the secretory power of the tubules is impaired although the renal blood flow is normal. It is obvious that both these factors can operate simultaneously to decrease the diodrast clearance. The only course open to the writer was to determine the quotient of the inulin clearance to the diodrast clearance, i.e. the filtration fraction. Figure 7 (v. p. 138) shows that this quotient is nearly always decreased when the inulin clearance falls to 90-95 ml per minute.

It is naturally possible that the cases are in varying stages as regards the process of recovery, so that one function has become normal or almost normal whereas others, or some of them, are still damaged. It is also possible that from the onset different partial functions are impaired to a varying degree. Both these factors must cooperate in order to give differences between the individual patients and the extent of the variations in the whole material thus becomes larger. This is also seen from Table XVI in which the standard deviation for the various determinations is considerably higher than for the normal material.

It is seen from the following that in acute nephritis the various clinical symptoms are more or less marked. Had the material been large enough it would have been most satisfactory to calculate the correlation. A *sine qua non* for such calculations is nevertheless that quantitative measures of the different symptoms can be obtained. This was possible in respect of, for example, the blood pressure and the urea



nitrogen, but not in respect of haematuria and proteinuria. The degree of haematuria must be determined quantitatively, for example according to Addis' method. An approximate calculation according to the frequently used terms «very abundant red blood corpuscles», «scanty», etc. gives, according to the majority of earlier workers, a very poor idea of the degree of haematuria.

When, however, we are dealing with proteinuria, the customary method of stating it in promille can not be used. This can naturally be subject to very considerable variations, depending on whether the determination was made on the 24-hour volume or on a random sample during this period and also on whether the volume of urine was large or small. The only accurate method is therefore to determine the amount of protein in grammes excreted per 24 hours. The data necessary to allow such calculations were not, however, available in the present investigation.

As pointed out earlier, the two obligatory symptoms of acute nephritis, i.e. proteinuria and haematuria, were always present. On the other hand, the facultative symptoms, elevation of the blood pressure, oedema or increase in the non-protein nitrogen were sometimes absent. With regard to the blood pressure, a special table has been made with the cases divided into different groups (Table XX). Since the blood pressure has «normally» a tendency to rise with increasing age, the number of cases in the age group over 46 years has been given in brackets. It is seen that in the majority of patients with an elevation of the blood pressure, this was moderate, provided that they were not over 46 years of age. In this respect as well the present material is in agreement with that of other workers, for example Rudebeck's (1946). In his summary of these questions he states: «a systolic blood pressure over 170 mm in the acute stage is rather uncommon, a pressure over 200 mm is very rare».

In order to illustrate how often the facultative symptoms mentioned occur, their frequency is given in Table XXI, in which the basis for the classification in the following into two sub-groups with regard to these symptoms has been used.

Table XX

Distribution of the blood pressure (in mm Hg) in cases with acute nephritis. The figures in brackets apply to individuals above 45 years of age (they are included in the figures without brackets).

Blood pressure mm Hg	71—80	81—90	91—100	101—110	111—120	121—130	131—140	141—150	151—160	161—170	171—180	181—190	191—200	> 201
Systolic.....	—	—	—	—	6 (1)	12	17	14 (2)	11 (2)	11 (2)	4	5 (2)	3 (1)	6 (6)
Diastolic .....	23 (1)	18 (2)	19 (5)	14 (2)	9 (2)	6 (4)	—	—	—	—	—	—	—	—

Table XXI

(Grouping of some symptoms in cases with acute nephritis.

Symptom	Systolic blood pressure		Diastolic blood pressure		Oedema		Non protein nitrogen	
	$\geq 160$ mm Hg	$< 160$ mm Hg	$\geq 100$ mm Hg	$< 100$ mm Hg	+	—	$> 40$ mg %	$\leq 40$ mg %
No. of cases	38	51	40	49	40	49	39	50
Per cent	43 %	57 %	45 %	55 %	45 %	55 %	44 %	56 %

It is seen from the table that somewhat less than 50 per cent of all the cases had an elevation of the blood pressure (both systolic and diastolic) as well as an increase of non protein nitrogen above the borderlines given. The same applies to the presence of oedema, which was manifested in 45 per cent (40 cases) whereas it was absent in 55 per cent (49 cases).

In view of the forementioned circumstances, the writer therefore considered it most suitable to divide the material into two sub-groups with regard to certain symptoms, i.e. those with slight and those with severe such symptoms, and to compare these two groups. The following classifications were made.

- A. 1. Cases with systolic blood pressure  $\geq 160$  mm Hg
- 2. Cases with systolic blood pressure  $< 160$  mm Hg
- B. 1. Cases with diastolic blood pressure  $\geq 100$  mm Hg
- 2. Cases with diastolic blood pressure  $< 100$  mm Hg
- C. 1. Cases with non protein nitrogen  $> 40$  mg per cent  
or urea nitrogen  $> 20$  mg per cent
- 2. Cases with non protein nitrogen  $\leq 40$  mg per cent  
or urea nitrogen  $\leq 20$  mg per cent
- D. 1. Cases with oedema
- 2. Cases without oedema
- E. 1. Cases apparently recovered within 3 months
- 2. Cases not recovered within 3 months

In classifications according to *clinical symptoms*, a first possibility is to compare patients with a certain symptom with those in whom it is absent or is of a lesser degree on a certain occasion. Whether a patient manifests a symptom or not on a given occasion is not, however, the only decisive factor. If, for example, a comparison is made between patients who are free from symptoms at a given moment and have thus improved in comparison with their condition at the onset of the disease, they can in turn be divided into two groups, i.e. those who at the onset were severely ill and those who were less severely ill. In other words, they are divided according to their symptoms on hospitalization. If this takes

place at the onset of the disease it naturally influences the comparison during its further course. In order to obtain a complete analysis of the material it is thus necessary to group the patients both according to the current symptoms and according to the course, i.e. to the earlier symptoms. The present material was not sufficiently large to permit such an analysis. The only remaining possibility was to compare patients with different symptoms at a given moment or to classify them according to the most pronounced symptom during the course of the illness. In practice, these are often the symptoms manifested at the earliest stage of the disease.

The writer therefore formulated the problem as follows: Are the results of functional tests in a patient with acute nephritis who, at the onset or later, but before the time of the investigation, manifested clinical symptoms of a mild or severe degree different from those found in a patient without such symptoms? Do the current symptoms at the time of the investigation alone determine the degree of functional impairment?

Figure 8 (v. page 141) shows that both these factors, i.e. the duration and severity of the disease and decrease of filtration rate are probably correlated. This question will be discussed later on. As clinical symptoms those given above are used, but not proteinuria or haematuria, which were primary criteria for the diagnosis of acute nephritis (see Discussion in the following).

As regards group E in the classification above, it was not however possible to take into consideration only the symptoms before the occasion of the investigation, but the time elapsing after it was also considered, provided that the investigation was made less than three months after the onset of the disease.

### The Systolic Blood Pressure

The borderline between the two sub-groups was fixed at 160 mm Hg. Since the majority of patients were under the age of 40 — more than 50 per cent were even under 30

years — we can be certain that such a borderline figure differentiates between patients who had an elevated blood pressure at the time of the investigation or prior to it and those in whom no such elevation was observed.

The first striking fact revealed by Table XXII, in which this grouping is used is that the level of the systolic blood pressure, although it showed a statistically significant difference between the two sub-groups, did not reach the stipulated level of 160 mm Hg, but remained at  $142.5 \pm 3.9$ . This depends on the grounds of classification used.

It is of interest, as is seen from the table, that the figure for the inulin clearance showed a statistically significant difference between the groups, and the creatinine clearance a probable difference ( $> 2.5$  times the standard error). The diodrast and urea clearances, as well as the other tests, showed no significant differences, although in several instances the differences were twice the standard error. This was also the case for the diastolic blood pressure which did not — as could have been expected — show a statistically significant difference. The urea nitrogen is somewhat strange in that the figure was lower in the group with the higher blood pressure, both in the male and the female group and for both sexes together. This can be due to random variation. Under conditions that are otherwise identical, the filtration, for example, should increase with a rise in blood pressure. On the other hand, an elevation of the blood pressure may indicate that the illness is more severe and the glomeruli more injured. These two tendencies can cause a displacement in the opposite direction. It is thus not so astonishing that a decrease in filtration is present at higher pressure whereas there is no demonstrable difference in the excretion of urea.

### The Diastolic Blood Pressure

100 mm Hg was fixed as the borderline between the two sub-groups for the same reasons as in respect of the systolic blood pressure. It must, however, be pointed out that this borderline is more diffuse than in the case of the latter,

Table XXII. Mean (M) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) for cases with acute nephritis, distributed according to the highest level of the systolic blood pressure if above or below 160 mm Hg.

Determination	Systolic blood pressure				
	$\geq 160$ mm Hg		< 160 mm Hg		
	Num- ber	M $\pm$ $\epsilon$ (M)	Num- ber	M $\pm$ $\epsilon$ (M)	
Men					
Inulin clearance .....	18	76.2 $\pm$ 7.4	33	100.6 $\pm$ 6.0	
Diodrast clearance .....	18	359.7 $\pm$ 27.2	33	406.0	
Filtration fraction .....	18	0.211 $\pm$ 0.013	33	0.247 $\pm$ 0.011	
Creatinine clearance .....	7	82.4	10	100.3 $\pm$ (12.8)	
Urea clearance .....	7	74.5	18	70.6 $\pm$ 7.6	
Effect. renal blood flow ....	18	610.2 $\pm$ 49.8	33	703.4	
Hæmatocrit .....	18	39.9 $\pm$ 1.4	33	41.6	
Proteinuria .....	18	0.39 $\pm$ 0.12	27	0.54	
Blood pressure {	systolic ....	18	132.5 $\pm$ 3.4	33	129.4
	diastolic ....	18	81.9 $\pm$ 2.6	33	81.1
Urea nitrogen, mg % ....	7	20.9	20	23.5 $\pm$ 3.0	
Women					
Inulin clearance .....	20	68.6 $\pm$ 6.9	18	93.6 $\pm$ 7.1	
Diodrast clearance .....	20	303.2 $\pm$ 26.4	18	344.2	
Filtration fraction .....	20	0.235 $\pm$ 0.018	18	0.272	
Creatinine clearance .....	13	73.3 $\pm$ (9.8)	6	120.8	
Urea clearance .....	9	48.6	10	55.7 $\pm$ (6.5)	
Effect. renal blood flow ....	20	498.6 $\pm$ 48.0	18	581.4	
Hæmatocrit .....	20	38.0 $\pm$ 1.4	18	40.6	
Proteinuria .....	20	0.74 $\pm$ 0.49	17	0.28	
Blood pressure {	systolic ....	20	151.5 $\pm$ 6.1	18	126.9 $\pm$ 1.9
	diastolic ....	20	92.0 $\pm$ 3.5	18	81.9 $\pm$ 2.7
Urea nitrogen, mg % ....	11	18.5 $\pm$ (1.8)	11	20.1	
Both sexes					
Inulin clearance .....	38	72.2 $\pm$ 5.0	51	98.2 $\pm$ 4.5	
Diodrast clearance .....	38	329.9 $\pm$ 19.2	51	384.2 $\pm$ 14.8	
Filtration fraction .....	38	0.223 $\pm$ 0.011	51	0.255 $\pm$ 0.009	
Creatinine clearance .....	20	76.5 $\pm$ 7.1	16	108.0 $\pm$ 9.7	
Urea clearance .....	16	59.9 $\pm$ 7.2	28	65.3	
Effect. renal blood flow ....	38	551.4 $\pm$ 35.5	51	660.4 $\pm$ 26.0	
Hæmatocrit .....	38	38.9 $\pm$ 1.0	51	41.3	
Proteinuria .....	38	0.57 $\pm$ 0.26	44	0.44	
Blood pressure {	systolic ....	38	142.5 $\pm$ 3.9	51	128.5 $\pm$ 1.4
	diastolic ....	38	87.2 $\pm$ 2.3	51	81.4 $\pm$ 1.5
Urea nitrogen, mg % ....	18	19.4 $\pm$ 1.5	31	22.3 $\pm$ 2.0	

<sup>1</sup> The standard error is only computed for one of the means, if the difference between them is below three times this error.

depending on how the diastolic pressure is determined (v. Chronic Glomerulonephritis).

Table XXIII gives the results according to this grouping. The same condition is found as in the foregoing table, i.e. the mean of the diastolic pressure did not reach the borderline fixed. The figures of both groups at the time of the test are given in order to give an idea of the size of the difference. The only statistically significant difference was that the inulin clearance for the sexes together and for women showed a lower value in the sub-group with diastolic pressure above 100 mm Hg. No such difference was found in the male group. This is probably connected with the fact that the women were on the average more severely ill and therefore the difference between the two sub-groups was more marked. A further result is that the filtration fraction and the systolic blood pressure also showed a statistically probable difference in the female sub-groups, whereas it was not apparent when a calculation was made for both sexes together. Nevertheless, the difference here, as in the case of the diodrast and creatinine clearances and the effective renal blood flow, was throughout slightly above twice the standard error and would probably have been statistically significant on a larger material.

It is, nevertheless, remarkable that the urea nitrogen level in the plasma was lower in those with the diastolic pressure  $\geq 100$  mm Hg. In other words, the position was the same as in the grouping according to the systolic pressure and the same conclusions can therefore be drawn.

### Non Protein Nitrogen or Urea Nitrogen

As is the case with all the other symptoms of renal disease to be discussed later in this paper, certain difficulties arose in grouping the patients according to two sub-groups, i.e. in choosing the limit between them. It was difficult to decide to which sub-group a patient should be assigned if the non protein nitrogen figure was within the normal variations but the urea nitrogen outside them or *vice versa*. If

Table XXIII. Mean ( $M$ ) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) for cases with acute nephritis, grouped according to the highest level of the diastolic blood pressure if above or below 100 mm Hg.

Determination	Diastolic blood pressure			
	$\geq 100$ mm Hg		< 100 mm Hg	
	Num- ber	$M \pm \epsilon(M)$	Num- ber	$M \pm \epsilon(M)$
M e n				
Inulin clearance .....	17	$81.3 \pm 7.1$	34	97.4
Diodrast clearance .....	17	$369.9 \pm 22.4$	34	399.6
Filtration fraction .....	17	$0.218 \pm 0.014$	34	0.242
Creatinine clearance .....	5	84.8	12	$96.3 \pm (11.5)$
Urea clearance .....	6	78.5	19	$69.5 \pm 7.3$
Effect. renal blood flow ....	17	$631.8 \pm 41.6$	34	689.9
Hæmatoerit .....	17	$40.4 \pm 1.6$	34	41.3
Proteinuria .....	17	$0.235 \pm 0.067$	28	$0.629 \pm 0.250$
Blood pressure { systolic ...	17	$130.6 \pm 2.9$	34	130.4
	17	$81.8 \pm 2.7$	34	81.2
Urea nitrogen, mg % .....	7	21.5	20	$23.3 \pm 3.0$
W o m e n				
Inulin clearance .....	23	$68.1 \pm 6.4$	15	$99.4 \pm 6.9$
Diodrast clearance .....	23	$304.2 \pm 25.7$	15	350.8
Filtration fraction .....	23	$0.232 \pm 0.016$	15	$0.284 \pm 0.012$
Creatinine clearance .....	12	$71.4 \pm (10.5)$	7	117.1
Urea clearance .....	11	$46.4 \pm (5.3)$	8	60.5
Effect. renal blood flow ....	23	$502.4 \pm 43.5$	15	592.1
Hæmatoerit .....	23	$38.6 \pm 1.3$	15	40.3
Proteinuria .....	23	$0.69 \pm 0.43$	14	0.26
Blood pressure { systolic ....	23	$146.7 \pm 5.7$	15	$129.3 \pm 2.9$
	23	$91.3 \pm 3.3$	15	$81.0 \pm 2.7$
Urea nitrogen, mg % .....	11	$18.4 \pm (1.7)$	11	20.1
B o t h   s e x e s				
Inulin clearance .....	40	$73.7 \pm 4.8$	49	$98.0 \pm 4.8$
Diodrast clearance .....	40	$332.1 \pm 18.1$	49	$384.6 \pm 15.7$
Filtration fraction .....	40	$0.226 \pm 0.011$	49	$0.255 \pm 0.009$
Creatinine clearance .....	17	$75.4 \pm 8.0$	19	$104.0 \pm 8.7$
Urea clearance .....	17	$57.7 \pm 7.3$	27	66.9
Effect. renal blood flow ....	40	$557.4 \pm 32.0$	49	$659.9 \pm 28.8$
Hæmatoerit .....	40	$39.4 \pm 1.0$	49	41.0
Proteinuria .....	40	$0.50 \pm 0.25$	42	0.51
Blood pressure { systolic ....	40	$139.9 \pm 3.7$	49	$130.1 \pm 1.7$
	40	$87.3 \pm 2.3$	49	$81.1 \pm 1.5$
Urea nitrogen, mg % .....	18	$19.6 \pm 1.4$	31	$22.2 \pm 2.1$

<sup>1</sup> v. foot note p. 129



several determinations were made at about the same time and the course of the illness gave no reason to suspect any change in the state of the patient, the decision was made according to the majority of these determinations. If the number of determinations in different directions was equal, the non protein nitrogen figure was assumed to be decisive. The reason was the one given in the following, i.e. that non protein nitrogen determinations are made as a matter of routine at the University Hospital in Upsala.

Table XXIV shows the figures according to this grouping. It is seen that it was still the inulin clearance figure alone that showed a lower value in the sub-group with an increase in urea or non protein nitrogen. The difference for both sexes together and for the men was statistically significant whereas the difference was only probable for the women. There was also a statistically probable difference between the sub-groups in both sexes with regard to the filtration fraction and the urea nitrogen. That the latter was not significant although it was the basis of the classification is conditioned by the same factor as when the material was classified according to the blood pressure. In other words, it was most frequently not a question of the figures found at the time of the investigation. For all the other tests, although there were no significant differences, there was a tendency throughout to decreased function in the sub-group with higher non protein nitrogen, with the exception of the blood pressure levels for which scarcely any difference could be shown. This fact is in good agreement with the conclusion that the level of the blood pressure is not decisive for the retention of non protein nitrogen (v. p. 128 and 130).

### Oedema

Very few of the patients manifested general oedema. The majority only showed a greater or lesser degree of facial oedema, puffiness or oedema of the eyelids, or latent oedema detected by repeated weighings.

Table XXV shows that here, too, a statistically significant

Table XXIV. Mean (M) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) for cases with acute nephritis, distributed according to the highest level of the non protein nitrogen if above or below 40 mg % (or urea nitrogen  $>$  or  $<$  20 mg %).

Determination	Non protein nitr. $>$ 40 mg % or Urea nitr. $>$ 20 mg %		Non protein nitr. $\leq$ 40 mg % or Urea nitr. $\leq$ 20 mg %	
	Num- ber	M $\pm$ $\epsilon$ (M)	Num- ber	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance .....	29	79.0 $\pm$ 6.6	22	109.2 $\pm$ 5.7
Diodrast clearance .....	29	360.0 $\pm$ 21.2	22	428.9 $\pm$ 19.3
Filtration fraction .....	29	0.215 $\pm$ 0.012	22	0.259 $\pm$ 0.013
Creatinine clearance .....	13	85.6 $\pm$ (8.4)	4	116.8
Urea clearance .....	14	56.5 $\pm$ (9.0)	11	91.0 $\pm$ (5.9)
Effect. renal blood flow ....	29	609.3 $\pm$ 39.7	22	751.2 $\pm$ 32.6
Hæmatocrit .....	29	39.8 $\pm$ 1.0	22	42.6 $\pm$ 1.0
Proteinuria .....	27	0.73 $\pm$ 0.25	18	0.11
Blood pressure { systolic ....	29	130.0 $\pm$ 2.6	22	131.1
	29	80.5 $\pm$ 2.1	22	82.5
Urea nitrogen, mg % .....	15	27.0 $\pm$ 3.8	12	17.7
Women				
Inulin clearance .....	10	59.6 $\pm$ (9.5)	28	87.9 $\pm$ 5.8
Diodrast clearance .....	10	277.6 $\pm$ (38.6)	28	338.6
Filtration fraction .....	10	0.227 $\pm$ (0.028)	28	0.261
Creatinine clearance .....	8	73.5	11	99.0 $\pm$ (12.1)
Urea clearance .....	4	40.8	15	55.4 $\pm$ 4.6
Effect. renal blood flow ....	10	440.1 $\pm$ (63.2)	28	572.7
Hæmatocrit .....	10	35.6 $\pm$ (2.1)	28	40.5
Proteinuria .....	10	1.26 $\pm$ (0.98)	27	0.26
Blood pressure { systolic ....	10	155.5 $\pm$ (11.5)	28	134.3
	10	93.5 $\pm$ (5.7)	28	85.0
Urea nitrogen, mg % .....	4	24.3	18	18.14 $\pm$ 0.93
Both sexes				
Inulin clearance .....	39	74.0 $\pm$ 5.6	50	97.3 $\pm$ 4.3
Diodrast clearance .....	39	338.8 $\pm$ 19.3	50	378.3
Filtration fraction .....	39	0.218 $\pm$ 0.011	50	0.260 $\pm$ 0.009
Creatinine clearance .....	21	81.0 $\pm$ 7.4	15	103.7 $\pm$ 10.5
Urea clearance .....	18	53.0 $\pm$ 7.3	26	70.5
Effect. renal blood flow ....	39	565.9 $\pm$ 35.3	50	651.2
Hæmatocrit .....	39	38.72 $\pm$ 0.97	50	41.46 $\pm$ 0.66
Proteinuria .....	37	0.87 $\pm$ 0.32	45	0.20
Blood pressure { systolic ....	39	136.5 $\pm$ 3.9	50	132.9
	39	83.8 $\pm$ 2.3	50	83.9
Urea nitrogen, mg % .....	19	26.45 $\pm$ 3.08	30	17.95 $\pm$ 0.71

<sup>1</sup> v. foot note p. 129

difference as regards inulin clearance in the two sub-groups was found for both sexes together. The conditions were the same in respect of the filtration fraction. In the remaining determinations the differences between the sub-groups were smaller and were only approximately twice the standard error in the case of the creatinine clearance and the effective renal blood flow, although the tendency was the same throughout in the other tests with lower values in those with oedema.

### The Duration

The last classification made by the writer was according to the duration of the acute nephritis, the borderline being fixed at three months. Attempts were first made to divide the material into several groups, but it was not sufficiently large for this purpose. Moreover, difficulties arose in many cases in classifying the patients according to more sharply-drawn limits. By dividing the material into two sub-groups with the forementioned limit greater accuracy in the classification was assured and it was then possible to study the effect on renal function in nephritis of short or long duration. On the other hand, it was not possible to study the question of how many or which patients would later manifest chronic nephritis since in the majority of cases the observation time was too short.

On perusal of Table XXVI it is seen that there were statistically significant lower values for the inulin and diodrast clearances and the effective renal blood flow in the group that had not recovered within three months. In respect of the other tests there were throughout signs of greater renal lesions in this group both in the sexes separately and together. The differences were twice the standard error or approaching this figure with the exception of the urea clearance for which the standard error and the difference were of the same order of magnitude.

Table XXV. Mean (M) and standard error of the mean<sup>1</sup> ( $\pm$ ) for cases with acute nephritis, distributed according to occurrence of œdema.

Determination	Cases			
	with œdema		without œdema	
	Num- ber	M $\pm$ $\varepsilon$ (M)	Num- ber	M $\pm$ $\varepsilon$ (M)
Men				
Inulin clearance .....	19	78.9 $\pm$ 7.7	32	99.8 $\pm$ 6.0
Diodrast clearance .....	19	369.7 $\pm$ 25.5	32	401.6
Filtration fraction .....	19	0.213 $\pm$ 0.014	32	0.246
Creatinine clearance .....	8	78.1	9	106.1
Urea clearance .....	9	65.0	16	75.4 $\pm$ 8.1
Effect. renal blood flow ....	19	628.4 $\pm$ 49.8	32	695.5
Hæmatocrit .....	19	39.7 $\pm$ 1.5	32	41.8
Proteinuria .....	18	0.73 $\pm$ 0.21	27	0.31
Blood pressure { systolic ....	19	131.3 $\pm$ 3.5	32	130.0
	19	81.8 $\pm$ 2.3	32	81.1
Urea nitrogen, mg % .. ...	9	25.1	18	21.7 $\pm$ 3.0
Women				
Inulin clearance .....	21	69.2 $\pm$ 5.4	17	94.3 $\pm$ 8.8
Diodrast clearance .....	21	313.9 $\pm$ 24.0	17	333.3
Filtration fraction .....	21	0.223 $\pm$ 0.014	17	0.289 $\pm$ 0.016
Creatinine clearance .....	9	76.1	10	99.2 $\pm$ (14.2)
Urea clearance .....	10	48.5 $\pm$ (4.8)	9	56.7
Effect. renal blood flow ....	21	513.6 $\pm$ 39.8	17	567.7
Hæmatocrit .....	21	38.5 $\pm$ 1.3	17	40.2
Proteinuria .....	21	0.70 $\pm$ 0.47	16	0.29
Blood pressure { systolic ....	21	144.3 $\pm$ 6.3	17	134.4
	21	89.3 $\pm$ 3.5	17	84.7
Urea nitrogen, mg % .....	10	17.4 $\pm$ (1.3)	12	20.9 $\pm$ (1.6)
Both sexes				
Inulin clearance .....	40	73.8 $\pm$ 4.6	49	97.9 $\pm$ 4.9
Diodrast clearance .....	40	340.4 $\pm$ 17.8	49	377.9
Filtration fraction .....	40	0.218 $\pm$ 0.010	49	0.261 $\pm$ 0.009
Creatinine clearance .....	17	77.1 $\pm$ 7.1	19	102.5 $\pm$ 9.5
Urea clearance .....	19	56.3 $\pm$ 6.1	25	68.7
Effect. renal blood flow ....	40	568.1 $\pm$ 32.5	49	651.2 $\pm$ 29.0
Hæmatocrit .....	40	39.1 $\pm$ 1.0	49	41.2
Proteinuria .....	39	0.72 $\pm$ 0.27	43	0.31
Blood pressure { systolic ....	40	138.1 $\pm$ 3.8	49	131.5
	40	85.8 $\pm$ 2.2	49	82.3
Urea nitrogen, mg % .....	19	21.0 $\pm$ 2.1	30	21.4 $\pm$ 1.9

<sup>1</sup> v. foot note p. 129

Table XXVI. Mean (M) and standard error of the mean<sup>1</sup>( $\epsilon$ ) for cases with acute nephritis, distributed according to the duration of the disease.

Determination	Recovered within 3 months		Not recovered within 3 months	
	Num- ber	M $\pm$ $\epsilon$ (M)	Num- ber	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance .....	28	101.0 $\pm$ 5.8	23	81.0 $\pm$ 7.8
Diodrast clearance .....	28	416.0 $\pm$ 15.7	23	357.7 $\pm$ 27.0
Filtration fraction .....	28	0.244 $\pm$ 0.013	23	0.221
Creatinine clearance .....	6	99.8	11	89.1 $\pm$ (12.0)
Urea clearance .....	17	75.2 $\pm$ 7.0	8	64.3
Effect. renal blood flow ....	28	724.8 $\pm$ 30.4	23	604.5 $\pm$ 47.5
Hæmatocrit .....	28	41.93 $\pm$ 0.92	23	39.91
Proteinuria .....	22	0.25 $\pm$ 0.12	23	0.70 $\pm$ 0.28
Blood pressure { systolic ....	28	128.4 $\pm$ 2.2	23	133.0
	28	80.5 $\pm$ 1.6	23	82.4
Urea nitrogen, mg % .....	18	21.9 $\pm$ 2.2	9	24.9
Women				
Inulin clearance .....	16	97.9 $\pm$ 6.4	22	67.8 $\pm$ 6.7
Diodrast clearance .....	16	368.3 $\pm$ 26.6	22	289.4 $\pm$ 22.3
Filtration fraction .....	16	0.273 $\pm$ 0.015	22	0.237
Creatinine clearance .....	8	113.6	11	69.8 $\pm$ (12.0)
Urea clearance .....	13	56.7 $\pm$ (4.4)	6	42.8
Effect. renal blood flow ....	16	617.4 $\pm$ 46.0	22	479.9 $\pm$ 38.3
Hæmatocrit .....	16	39.9 $\pm$ 1.2	22	38.8
Proteinuria .....	15	0.160 $\pm$ 0.074	22	0.777 $\pm$ 0.445
Blood pressure { systolic ....	16	135.3 $\pm$ 3.6	22	143.2
	16	83.4 $\pm$ 2.0	22	90.0 $\pm$ 3.8
Urea nitrogen, mg % .....	15	18.09 $\pm$ 0.92	7	21.79
Both sexes				
Inulin clearance .....	44	99.9 $\pm$ 4.3	45	74.5 $\pm$ 5.2
Diodrast clearance .....	44	398.6 $\pm$ 14.1	45	324.3 $\pm$ 18.1
Filtration fraction .....	44	0.255 $\pm$ 0.010	45	0.229 $\pm$ 0.010
Creatinine clearance .....	14	107.7 $\pm$ (7.4)	22	79.5 $\pm$ 8.5
Urea clearance .....	30	67.2 $\pm$ 4.6	14	55.1 $\pm$ (9.3)
Effect. renal blood flow ....	44	685.7 $\pm$ 26.4	45	543.6 $\pm$ 31.8
Hæmatocrit .....	44	41.18 $\pm$ 0.73	45	39.36
Proteinuria .....	37	0.211 $\pm$ 0.079	45	0.740 $\pm$ 0.258
Blood pressure { systolic ....	44	130.9 $\pm$ 2.0	45	138.0 $\pm$ 3.3
	44	81.6 $\pm$ 1.3	45	86.1 $\pm$ 2.3
Urea nitrogen, mg % .....	33	20.1 $\pm$ 1.3	16	23.5 $\pm$ 3.3

<sup>1</sup> v. foot note p. 129

### *Summary and Discussion*

An account has been given in the foregoing of the numerical results obtained but no closer discussion of their implication has been entered into. All the patients obviously manifested the obligatory symptoms, i.e. proteinuria and haematuria, and the facultative symptoms — elevation of the blood pressure, increase in non protein nitrogen or urea nitrogen and oedema — occurred in 43 to 45 per cent of the cases (Table XXI, p. 125).

In the study of the material an analysis was first made of how large a proportion manifested pathologically decreased figures for the various tests (Table XVIII) and further a calculation of how often two different tests showed simultaneously decreased figures (Table XIX).

As regards the first of these analyses, it was found that approximately 50 per cent of the cases had a decreased inulin clearance and the conditions were practically the same in the case of creatinine clearance. The number with pathological figures was smaller in the other tests, particularly in respect of urea clearance and urea nitrogen. The reason that a larger proportion of the material did not manifest definitely pathological figures is obviously to be sought in the fact that many of the patients only suffered from mild nephritis. This can also be deduced from the fact that only 43 to 45 per cent of the cases had facultative symptoms.

A calculation of the correlation between the various tests showed not only that those tests which are referable to one and the same renal function were simultaneously depressed but also that there was a correlation between tests relating to different processes in the kidney. The latter fact would appear to indicate that several parts of the nephron are damaged.

In addition, a comparison has been made between the results of the various tests in the nephritic material as a group and the normal material. Briefly, the results were as follows.

Statistically significant differences from the normal were

obtained for all the figures with the exception of urea nitrogen and the systolic blood pressure for which the differences were only approximately twice the standard error and for the urea clearance for which, however, it was statistically probable. As a typical finding in this disease, the writer — as did earlier workers — particularly, Steinitz (1941) and Hilden (1945), observed that although all the clearance figures were decreased, that of inulin was more decreased than that of diodrast and the filtration fraction was thus lower than normal. Is this fact of practical importance

F. F.

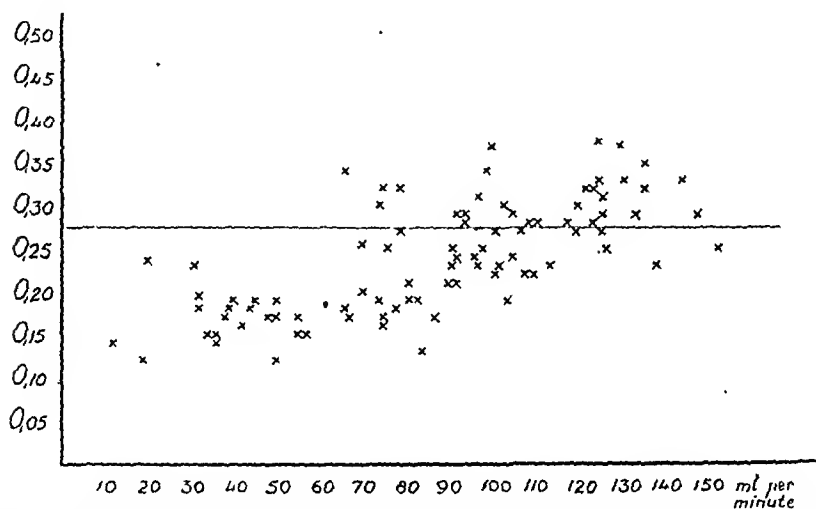


Fig 7. The filtration fractions (F.F.) tabulated according to increasing filtration rates (ml per min) in acute nephritis. The horizontal line indicates the normal value of the filtration fraction.

when judging an individual case? In order to answer this question, a survey was made of the entire material and there the figures for the filtration fraction were combined with rising figures for inulin clearance (v. fig. 7). It is seen that there is a general tendency to a lower filtration fraction the lower the inulin clearance. Moreover, it can be seen that at inulin clearances below 60 ml per minute, all the 22 patients had a filtration fraction far below normal. It was between 0.15-0.20 for the majority as compared with 0.275 for the normal material. At inulin clearances below

90 ml per minute, all the 41 patients had a lowered filtration fraction, with the exception of four, who had figures between 0.30-0.35. When the filtration rose above 90 ml per minute, approximately one half of the cases had a lowered and the rest a raised filtration fraction. The diagram thus shows that the majority of patients with a filtration figure between 90 and 120 ml per minute had a filtration fraction below the normal level, whereas the opposite occurred in those with an inulin clearance of 120-155 ml per minute, i.e. a raised figure for the majority.

To sum up the information given by this diagram, it can be stated that *acute* nephritis with a marked decrease in the filtration rate almost without exception exhibits a low filtration fraction and the diagnosis is thus confirmed. On the other hand, the filtration fraction affords very little information concerning the possibilities of recovery or whether there is a tendency to a chronic form of the disease. Nor does it help us to assess the future duration of the disease. Certain information regarding the last-mentioned can nevertheless be obtained from the degree of disturbance in renal function (v. the following).

When the manner in which this disturbance takes place is to be discussed in acute nephritis, reference is made to the chapter »General Discussion» in the present paper, where this problem is dealt with in connexion with the other groups of diseases investigated.

The writer came to the following conclusions after grouping the material according to different clinical data such as blood pressure, oedema, level of non protein nitrogen, and with regard to the duration of the disease. In the different groups two sub-groups are set up, i.e. one with more and one with less pronounced symptoms. In the sub-groups with more pronounced symptoms and longer duration respectively, renal function is more impaired. For some of the groups there are significant differences between the two sub-groups in the results of the tests but such differences are found throughout in respect of the inulin clearance. The tendency is otherwise practically identical for the other figures, i.e.



poorer function accompanying more severe symptoms or longer duration, although the differences are not statistically significant or even probable in the majority of cases. This was naturally expected *a priori*, but the significance of these observations is that they afford further proof of the value of clearance determinations when the nature and the extent of renal damage are to be estimated. As a complement to clinical examinations, these clearance determinations afford a possibility of registering — in a way easier than any other — changes towards improvement or deterioration.

The forementioned applies to the diagnosis of acute nephritis and the possibility of determining the degree of renal impairment by means of clearance determinations. What are then the possibilities of obtaining an idea of the prognosis or the duration of the disease on the basis of these tests? In answering the first part of the question no statement can be made: the prognosis *quoad vitam* was good in all the cases investigated. Since, however, it was not possible to follow up the cases for a long time — the longest period being four years — the question of the prognosis as regards recovery must be left, on the whole, unanswered. It is scarcely possible from fig. 7 to envisage how those cases that fully recover can be separated from those that pass over into a chronic stage. The latter should probably be sought among those showing the greatest impairment of renal function at the investigation. Here, however, the figures for the filtration fraction are so uniform that it would be difficult to obtain any information even if we could anticipate the future condition of the patient after, for example, ten or twenty years. On the other hand, as mentioned earlier, there are certain possibilities of estimating the duration of the disease on the basis of the functional tests. In order to demonstrate this, the writer drew a diagram (fig. 8) based on the following classifications: I = cases that showed increased values in the non protein nitrogen (or proportionate rises in the urea nitrogen) at the investigation. II = cases that at the time of the investigation had normal figures for non protein nitrogen and/or urea nitrogen, but at the onset of the disease

Inulin cl.

I

II

III

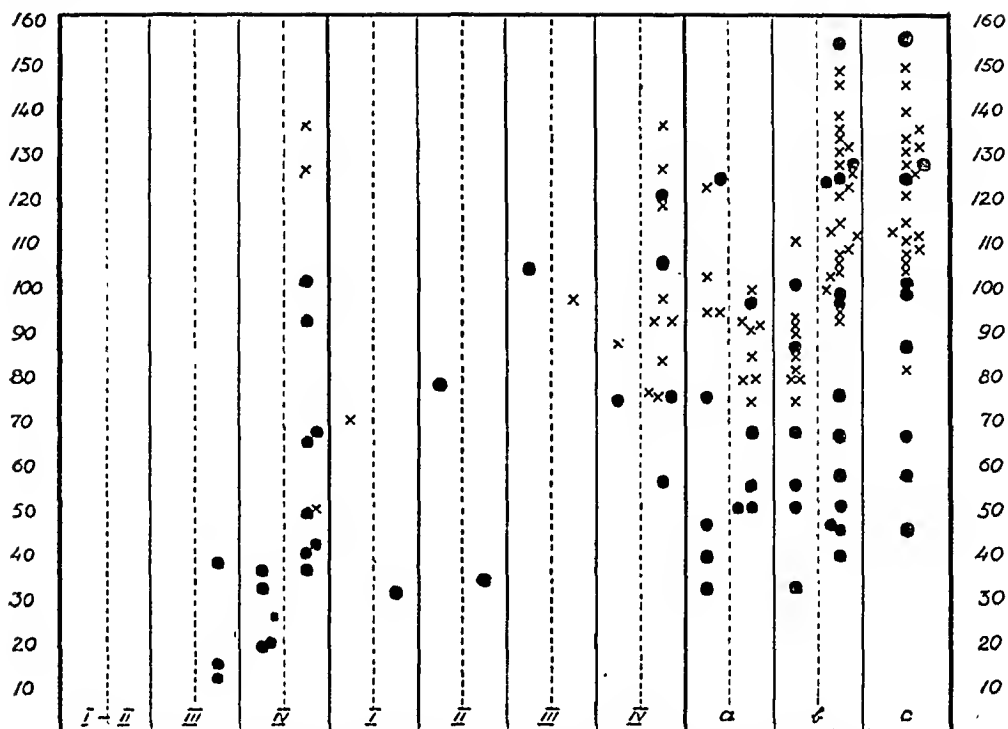


Fig 8. Diagram showing the relation between inulin clearance and various clinical symptoms with regard to the time for recovery in acute nephritis. Explanation in the text (p. 140—41).

or during its course (but before the investigation) had raised figures for one or other or both of these determinations. Four sub-groups were made within each of these groups according to the level of the non protein nitrogen. (I:  $> 100$  mg %, II:  $100-81$  mg %, III:  $80-61$  mg % and IV:  $60-41$  mg %.) Within each of these sub-groups, the cases were classified according to whether they had normal blood pressure or not at the time of the investigation (blood pressure  $\geq 160/90$  on the left and pressure  $< 160/90$  on the right side of the dotted line). Group III included patients who, neither at the time of the investigation nor earlier, showed any rise in non protein nitrogen. There were three sub-groups here, i.e. according to the level of the blood pressure at the time of the investigation ( $\leq 160/90$  mm Hg respectively) [a], oedema or absence of oedema [b], and lastly those who in addition to haema-

turia and proteinuria showed no such "peripheral" signs of acute nephritis [c].

The inulin clearance figures are given in this diagram (fig. 8), the cases that recovered within three months being denoted with X and those that were apparently not then recovered but concerning whose future fate information was not always available with ●. The following information can be obtained from this diagram. In group I, i.e. those with a rise in non protein nitrogen, the duration was more than three months in 15 of the 18 cases. Two of these 18 cases had normal inulin clearance figures at the time of the investigation and both were clinically recovered within three months. In group II, i.e. the patients with retention of non protein nitrogen earlier but not at the time of the investigation, it is seen that the lower the inulin clearance the greater are the prospects of a duration of more than three months. The conditions are the same in group III. It is thus seen that when the filtration values were below 70 ml/min, the duration of the disease had been more than three months with the exception of one case. This was independent of whether the patient was hypertensive or not, whether he was or was not oedematous or if he showed no other symptoms than the positive findings in the urine, i.e. haematuria and proteinuria.

The only conclusions that can be drawn from the foregoing are the following. The duration of the disease is in the majority of cases more than three months for azotaemic patients. The results of renal functional tests give more accurate aid than any clinical symptom in deciding whether the patient can recover from nephritis within three months or not, provided that filtration is below 70 ml/min. Of the 27 patients who showed a lower filtration figure, only one had apparently recovered within three months. With higher filtration figures, the prospect of a shorter duration is considerably greater, and the majority of cases recovered within this period. If this is confirmed on a larger material — and in view of the unequivocal figures there is no reason to doubt this — the determination of the filtration rate can be considered

as a good diagnostic aid, at any rate as regards the duration of the disease. In other words: a filtration value below 70 ml/min in all probability means a duration of at least three months. On the other hand, it must once more be emphasized that the question of whether the illness will be chronic or not cannot be answered by means of such a determination.

## Chronic Glomerulonephritis

The writer's material consisted of 48 patients, 25 men and 23 women, suffering from chronic nephritis. In handling this material, the same analytical method was used, as for acute nephritis.

An account is first given of the age-distribution of the material. A comparison is then made between the chronic nephritics and the normal material both as a group and individually in respect of the known borderlines,  $2\sigma$  and  $3\sigma$  respectively. A calculation is then made of the correlation between the different tests as well as between the various symptoms and the results of these tests. The distribution according to age is seen from the general survey of the material (Table XI, p. 95).

The results of all the tests made are given in tabular form (Table XXVII) and it can be seen that all the mean figures for both men and women are considerably lower than for the normal material with the obvious exception of those for systolic and diastolic blood pressure and for urea nitrogen, which are higher. The filtration fraction is, however, of the same order of magnitude as in the normal material. The significance of the latter fact will be discussed later (p. 164). The standard deviation of the various figures is naturally considerably greater than in the normal material since this material consisted of individuals in different stages of chronic nephritis, from relatively early stages to the final uraemic one. All the differences between the mean figures for the normal cases and those for the chronic nephritics are statistically significant with the exception of the forementioned filtration fraction (Table XXVIII).

Table XXVII

Mean (M) standard error of the mean ( $\epsilon$ ) and standard deviation ( $\sigma$ ) in different tests for men and women with chronic nephritis.

Determination	Men			Women			Both sexes		
	Num- ber	M $\pm$ $\epsilon$ (M)	$\sigma$	Num- ber	M $\pm$ $\epsilon$ (M)	$\sigma$	Num- ber	M $\pm$ $\epsilon$ (M)	$\sigma$
Inulin clearance .....	25	43.7 $\pm$ 5.6	27.9	23	44.9 $\pm$ 5.8	27.9	48	44.3 $\pm$ 4.0	27.6
Diodrast clearance .....	25	173.4 $\pm$ 23.3	116.4	23	172.3 $\pm$ 21.4	102.6	48	172.9 $\pm$ 15.7	108.8
Filtration fraction .....	25	0.270 $\pm$ 0.016	0.079	23	0.280 $\pm$ 0.018	0.084	48	0.275 $\pm$ 0.012	0.081
Creatinine clearance .....	7	43.4	—	8	57.1	—	15	50.7 $\pm$ 8.8	34.2
Urea clearance .....	19	35.5 $\pm$ 3.9	16.9	12	41.8 $\pm$ (7.2)	25.0	31	37.9 $\pm$ 3.6	20.3
Effect. renal blood flow ...	25	292.0 $\pm$ 39.9	199.5	23	269.7 $\pm$ 35.0	167.8	48	281.3 $\pm$ 26.5	183.4
Hematocrit .....	25	38.0 $\pm$ 1.5	7.5	23	34.5 $\pm$ 1.5	7.4	48	36.3 $\pm$ 1.1	7.6
Proteinuria .....	25	1.59 $\pm$ 0.53	2.64	23	2.50 $\pm$ 0.83	4.00	48	2.03 $\pm$ 0.48	3.35
Blood pressure {systolic ..	25	176.2 $\pm$ 6.7	33.7	23	173.5 $\pm$ 7.0	38.6	48	174.9 $\pm$ 4.8	33.3
{diastolic ..	25	108.8 $\pm$ 4.7	23.4	23	107.2 $\pm$ 4.7	22.6	48	108.0 $\pm$ 3.3	22.8
Urea nitrogen, mg % .....	20	33.6 $\pm$ 4.5	20.0	16	30.8 $\pm$ 3.1	12.4	36	32.3 $\pm$ 2.8	16.9

Table XXVIII

Differences between normal cases and cases with chronic nephritis in regard to different tests. A minus difference signifies that the normal figure is larger, a plus difference signifies the contrary.

Determination	Men	Women	Both sexes
Inulin clearance . . . . .	- 80.4 $\pm$ 6.0	- 73.9 $\pm$ 6.5	77.9 $\pm$ 4.4
Diodrast clearance . . . .	275.5 $\pm$ 24.3	- 264.1 $\pm$ 23.6	- 271.3 $\pm$ 16.7
Filtration fraction . . . . .	- 0.007 $\pm$ 0.017	+ 0.007 $\pm$ 0.019	- 0.001 $\pm$ 0.012
Creatinine clearance . . . .	- 73.6 $\pm$ 16.1	- 93.3 $\pm$ 13.1	90.2 $\pm$ 10.2
Urea clearance . . . . .	- 46.9 $\pm$ 6.5	- 33.4 $\pm$ 10.3	- 42.4 $\pm$ 5.3
Effect. renal blood flow . .	560.9 $\pm$ 41.4	- 493.2 $\pm$ 38.0	- 537.6 $\pm$ 28.5
Hamatocrit . . . . .	9.4 $\pm$ 1.6	8.3 $\pm$ 1.7	- 9.4 $\pm$ 1.2
Blood pressure {	+ 45.8 $\pm$ 6.8	+ 43.0 $\pm$ 7.2	+ 44.5 $\pm$ 4.9
systolic . . . . .			
diastolic . . . . .	+ 33.0 $\pm$ 4.7	+ 30.2 $\pm$ 4.9	+ 31.8 $\pm$ 3.3
Urea nitrogen, mg % . . .	+ 16.4 $\pm$ 4.6	+ 12.0 $\pm$ 3.4	+ 14.7 $\pm$ 2.9

It is of particular interest in this connexion to investigate to what extent the figures obtained fall outside the normal range of variation. A calculation of this has been made in Table XXXI for those tests which showed no significant difference between the sexes in the normal material. If the borderline is fixed at  $3\sigma$ , 92 per cent show decreased values for the inulin and diodrast clearances and a somewhat smaller percentage for the creatinine clearance. As was the case for acute nephritis, the percentage is smallest for the urea clearance and urea nitrogen. The number of cases with a decreased filtration fraction is considerably smaller than those with a decrease in the inulin and diodrast clearances. This is linked with the state of the filtration fraction in impaired renal function (v. Summary and Discussion).

The mutual relation between the various tests is seen in Table XXIX, in which, in the same way as in acute nephritis, a calculation is made of the expected and observed figures. The expected figures are obviously considerably higher here than the corresponding ones for acute nephritis, since in this material of chronic nephritis there is a far larger number of cases with very reduced renal function (v. Table XXXI).

Table XXIX

Expected and observed values (in per cent) of cases with chronic nephritis outside certain limits for normal values (2  $\sigma$  and 3  $\sigma$ ) in pairs of different tests<sup>1</sup>.

Determination	Number	Expected value, %		Observed value, %	
		2 $\sigma$	3 $\sigma$	2 $\sigma$	3 $\sigma$
Inulin clearance — Diodrast clearance	48	86 $\pm$ 5.0	85 $\pm$ 5.2	90	90
Inulin clearance — Filtration fraction ..	48	37 $\pm$ 7.0	29 $\pm$ 6.5	38	29
Inulin clearance — Creatinine clearance	15	80 $\pm$ 10.3	74 $\pm$ 11.3	87	80
Inulin clearance — Urea clearance ....	31	63 $\pm$ 8.7	27 $\pm$ 8.0	68	29
Inulin clearance — Urea nitrogen, mg %	36	56 $\pm$ 8.3	43 $\pm$ 8.3	61	47
Diodrast clearance — Filtration fraction ..	48	38 $\pm$ 7.0	29 $\pm$ 6.5	38	29
Diodrast clearance — Creatinine clearance	15	82 $\pm$ 9.9	74 $\pm$ 11.3	87	80
Diodrast clearance — Urea clearance ....	31	64 $\pm$ 8.6	27 $\pm$ 8.0	65	29
Diodrast clearance — Urea nitrogen, mg %	36	57 $\pm$ 8.3	43 $\pm$ 8.3	58	44
Filtration fraction — Creatinine clearance	15	35 $\pm$ 12.3	25 $\pm$ 11.2	33	13
Filtration fraction — Urea clearance ....	31	27 $\pm$ 8.0	9 $\pm$ 5.1	35	16
Filtration fraction — Urea nitrogen, mg %	36	24 $\pm$ 7.1	15 $\pm$ 6.0	28	22
Creatinine clearance — Urea clearance ....	6	59	23	50	50
Creatinine clearance — Urea nitrogen, mg %	18	53 $\pm$ 11.8	38 $\pm$ 11.4	38	38
Urea clearance — Urea nitrogen, mg %	31	41 $\pm$ 8.8	14 $\pm$ 6.2	52	29

<sup>1</sup> The expected values are obtained simply as a product of the frequency of the respective tests (v. Table XXXI) and should apply if there was only a random combination.

The figures obtained empirically agree with the calculated and expected figures. There are no significant differences. The standard error is, however, large owing to the small size of the material. It is possible that in a larger material a correlation could be shown by means of a corresponding comparison. This was not possible in the present writer's material.

Reference is made to the account in Chapter III for the criteria required for the diagnosis of chronic nephritis. It need only be recalled here that proteinuria and haematuria or other pathological changes in the urinary sediment are obligatory symptoms. The facultative symptoms are retinal

Table XXX

Grouping of some symptoms in cases with chronic nephritis.

Symptom	Systolic blood pressure $\geq 190$ mm Hg < 190 mm Hg	Diastolic blood pressure $\geq 100$ mm Hg < 100 mm Hg	Retin. changes 0 — II III — IV	Non protein nitrogen		Hæmoglobin	
				> 40 mg %	$\leq 40$ mg %	< 75 %	$\geq 75$ %
No. of cases	18	30	32	16	31	18	28
Per cent	38 %	62 %	67 %	33 %	63 %	37 %	61 %

Table XXXI

Number and percentage of individuals with chronic nephritis falling outside the normal limits calculated according to  $2\sigma$  and  $3\sigma$  respectively.

Determination	No.	$2\sigma$	$3\sigma$
Inulin clearance .....	48	44 (92 %)	44 (92 %)
Diodrast clearance .....	48	45 (94 %)	44 (92 %)
Filtration fraction .....	48	19 (40 %)	15 (31 %)
Creatinine clearance .....	15	13 (87 %)	12 (80 %)
Urea clearance .....	31	21 (68 %)	9 (29 %)
Urea nitrogen, mg. % .....	36	22 (61 %)	17 (47 %)



changes, elevation of the blood pressure, raised non protein nitrogen and anaemia. The frequency of these symptoms is seen in Table XXX.

In order to obtain an idea of the extent to which the decrease in renal function can be judged by the actual clinical symptoms, the following survey was made. Since, however, the material is small the number of cases did not always allow a calculation for men and women separately. Calculations were therefore also made throughout for both sexes together.

The material was divided into the following sub-divisions:

- A. 1. Cases with retinal changes I-II.
- 2. Cases with retinal changes III-IV.
- B. 1. Cases with systolic blood pressure  $\geq 190$  mm Hg.
- 2. Cases with systolic blood pressure  $< 190$  mm Hg.
- C. 1. Cases with diastolic blood pressure  $\geq 100$  mm Hg.
- 2. Cases with diastolic blood pressure  $< 100$  mm Hg.
- D. 1. Cases with non protein nitrogen  $> 40$  mg per cent  
or urea nitrogen  $> 20$  mg per cent.
- 2. Cases with non protein nitrogen  $\leq 40$  mg per cent  
or urea nitrogen  $\leq 20$  mg per cent.
- E. 1. Cases with anaemia, Hb  $< 75$  per cent.
- 2. Cases without anaemia, Hb  $\geq 75$  per cent.

This classification is also based on the fact that the prognosis for this disease differs in the two sub-divisions in groups A-E and it was therefore from this point of view as well considered of interest to investigate the state of renal function in the various groups. In the survey of this material (Table XXXII) all the cases have therefore been shown classified according to the groups in the foregoing and distributed according to the age-groups from  $\leq 20$  to  $> 71$  years. The number of deaths in the various groups is given in brackets as a rough contrast to the prognosis.

### Retinal Changes

There are various basic methods for the classification of retinal changes. The present writer has classified them according to the criteria set up by Keith (1933) into the

Table XXXII. The material of chronic nephritis tabulated according to age and various symptoms. The figures in brackets indicate the number of deaths.

Age, years	All cases	Retinal changes		Syst. blood pressure mm Hg		Diast. blood pressure mm Hg		Non prot. nitrogen mg %		Hæmoglobin %			
		0 — II	III — IV	≥ 190	< 190	≥ 100	< 100	> 40	< 40	< 75	≥ 75		
Men													
		A <sub>1</sub>	A <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	C <sub>1</sub>	C <sub>2</sub>	D <sub>1</sub>	D <sub>2</sub>	E <sub>1</sub>	E <sub>2</sub>		
≤ 20	0	—	—	—	—	—	—	—	—	—	—	—	—
21 — 30	7 (2)	5 (1)	2 (1)	3 (1)	4 (1)	6 (2)	1	4 (2)	—	1 (1)	—	6 (1)	—
31 — 40	4 (1)	3	1 (1)	1	3 (1)	3 (1)	1	4 (1)	3	—	—	4 (1)	—
41 — 50	4 (2)	2	2 (2)	2 (2)	2	2 (2)	2	2 (2)	2	—	—	4 (2)	—
51 — 60	5 (3)	2 (1)	3 (2)	3 (2)	2 (1)	3 (2)	2 (1)	5 (3)	—	2 (2)	—	2 (1)	—
61 — 70	4 (1)	2 (1)	2	1	3 (1)	2	2 (1)	3 (1)	1	3 (1)	—	1	—
> 71	1	1	—	—	1	—	1	1	—	—	—	1	—
Total	25 (9)	15 (3)	10 (6)	10 (5)	15 (4)	16 (7)	9 (2)	19 (9)	6	6 (4)	—	18 (5)	—
Women													
		A <sub>1</sub>	A <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	C <sub>1</sub>	C <sub>2</sub>	D <sub>1</sub>	D <sub>2</sub>	E <sub>1</sub>	E <sub>2</sub>		
≤ 20	3 (1)	2	1 (1)	2 (1)	1	2 (1)	1	2 (1)	1	1	—	2 (1)	—
21 — 30	6 (2)	4 (2)	2	1	5 (2)	5 (1)	1 (1)	4 (2)	1	3 (2)	—	3	—
31 — 40	2	2	—	1	1	2	—	2	—	—	—	1	—
41 — 50	3 (1)	2	1 (1)	1 (1)	2	2 (1)	1	3 (1)	—	2	—	1 (1)	—
51 — 60	3 (2)	—	3 (2)	2 (1)	1 (1)	1	2 (2)	2 (1)	1 (1)	2 (2)	—	1	—
61 — 70	4 (2)	4 (2)	—	—	4 (2)	2 (2)	2	2 (1)	2 (1)	1 (1)	—	1 (1)	—
> 71	2	2	—	1	1	2	—	2	—	1	—	1	—
Total	23 (8)	16 (4)	7 (4)	8 (3)	15 (5)	16 (5)	7 (3)	17 (6)	5 (2)	11 (5)	—	10 (3)	—
Total no.	48	31	17	18	30	32	16	36	11	17	—	28	—
Dead	17	7	10	8	9	12	5	15	2	9	—	8	—
% deaths	35.4	22.6	58.8	44.4	30.0	37.5	31.2	41.7	18.2	53.0	—	28.6	—

following four groups: I. Somewhat narrower blood vessels than normal, possibly slight changes in calibration. II. Narrow vessels, pronounced changes in calibration. III. Haemorrhages and exudate, mild papilloedema. IV. Pronounced papilloedema and usually the same symptoms as in III as well. No retinal changes are denoted with 0. This classification was originally intended for use in essential hypertension, but there is obviously nothing to prevent its use in chronic glomerulonephritis since the retinal changes are in principle of the same nature in both diseases.

In order to make possible a comparison between the state of renal function and retinal changes, the writer was obliged to amalgamate groups 0-II and to compare them with the group of severe changes in the fundi, which includes Keith's groups III-IV.

Table XXXIII shows the respective figures for men and women separately and for both sexes together. There is hardly any reason why the sexes should behave differently in respect of the symptom in question, and the figures are given merely for the sake of completeness. The figures for both sexes only will be discussed.

As is seen from the table there were only statistically significant differences between the sub-groups in respect of the systolic and the diastolic blood pressure, i.e. higher values in the group III-IV. As regards the urea nitrogen the difference also was significant. In order to get a reliable standard error, however, for the group with retinal changes III-IV a special method of computing this standard error was used. It was assumed that if there was no difference between the groups, there also should be about the same standard deviation for them. Consequently, the standard deviation for the group with retinal changes I-II was used for computing the standard error of the mean for the group with retinal changes III-IV; this because that group was too small to give a reliable standard deviation. In the following the same method was used whenever a similar situation arose.

In all the other tests, with the exception of proteinuria and the filtration fraction in which no difference could be

Table XXXIII.

Mean (M) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) in cases with chronic nephritis distributed according to retinal changes.

Determination	Retinal changes			
	0 — II		III — IV	
	Num- ber	M $\pm$ $\epsilon$ (M)	Num- ber	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance .....	15	56.3 $\pm$ 7.4	10	23.4 $\pm$ (3.9)
Diodrast clearance .....	15	224.8 $\pm$ 30.4	10	96.4 $\pm$ (19.0)
Filtration fraction .....	15	0.262 $\pm$ 0.017	10	0.281 $\pm$ (0.031)
Creatinine clearance .....	2	81.0	6	26.8
Urea clearance .....	13	41.2 $\pm$ 4.5	6	23.2
Effect. renal blood flow ....	15	383.0 $\pm$ 52.2	10	155.6 $\pm$ (28.3)
Hæmatocrit .....	15	39.1 $\pm$ 2.1	10	35.3 $\pm$ (2.1)
Proteinuria .....	15	2.15 $\pm$ 0.83	10	1.65 $\pm$ (0.97)
Blood pressure { systolic ....	15	161.3 $\pm$ 6.0	10	204.1 $\pm$ (11.6)
diastolic ...	15	98.7 $\pm$ 4.4	10	126.4 $\pm$ (7.3)
Urea nitrogen, mg % .....	14	24.3 $\pm$ (1.8)	6	55.2
Women				
Inulin clearance .....	16	44.2 $\pm$ 7.0	7	46.6
Diodrast clearance .....	16	168.3 $\pm$ 25.2	7	181.7
Filtration fraction .....	16	0.289 $\pm$ 0.022	7	0.261
Creatinine clearance .....	5	69.6	3	36.3
Urea clearance .....	10	37.0 $\pm$ (7.8)	2	66.0
Effect. renal blood flow ....	16	263.4 $\pm$ 43.1	7	284.1
Hæmatocrit .....	16	33.8 $\pm$ 1.8	7	36.1
Proteinuria .....	16	2.6 $\pm$ 1.0	7	2.3
Blood pressure { systolic ....	16	161.9 $\pm$ 6.3	7	200.0
diastolic ...	16	100.6 $\pm$ 4.1	7	122.1
Urea nitrogen, mg % .....	12	31.8 $\pm$ (4.0)	4	27.8
Both sexes				
Inulin clearance .....	31	50.1 $\pm$ 5.1	17	32.4 $\pm$ 5.5
Diodrast clearance .....	31	195.6 $\pm$ 20.0	17	131.5 $\pm$ 22.8
Filtration fraction .....	31	0.276 $\pm$ 0.014	17	0.273 $\pm$ 0.021
Creatinine clearance .....	7	72.9	9	30.0
Urea clearance .....	23	39.3 $\pm$ 4.2	8	33.9
Effect. renal blood flow ....	31	321.3 $\pm$ 34.8	17	208.5 $\pm$ 33.9
Hæmatocrit .....	31	36.3 $\pm$ 1.4	17	35.6 $\pm$ 1.7
Proteinuria .....	31	2.38 $\pm$ 0.64	17	1.90 $\pm$ 0.84
Blood pressure { systolic ....	31	161.6 $\pm$ 4.3	17	202.5 $\pm$ 8.7
diastolic ...	31	99.7 $\pm$ 2.9	17	124.7 $\pm$ 6.0
Urea nitrogen, mg % .....	26	27.8 $\pm$ 2.2	10	44.2 $\pm$ (3.5)

<sup>1</sup> v. foot note p. 129

found, there was, nevertheless, a tendency throughout to greater functional impairment in the group III-IV as compared with the group 0-II. This difference was throughout slightly more than twice the standard error. Had the material been larger, it is very probable that this tendency would have been significant.

### The Systolic Blood Pressure

The basis on which the classification was made in this group is naturally open to discussion on various grounds. The aim of the present writer was to investigate whether a considerable elevation of the blood pressure had any relation to the actual renal function. The material was not sufficiently large to give the figures for groups with varying degrees of hypertension, which would otherwise obviously have been desirable. A survey of the situation is given in fig 10. It was therefore necessary to fix some value as a borderline for high blood pressure. This naturally implies considerable difficulties, particularly since the present material comprised individuals in such varying age-groups. Somewhat over 50 per cent of the cases were less than 50 years of age. 190 mm Hg was chosen for the following reasons. At or above this figure the blood pressure is always considered as pathologically raised. Moreover, this limit was suitable to use in the present material for a division into groups each containing half the number of cases.

It is seen from Table XXXIV that there was no statistically significant difference between the two sub-groups except the obvious one in regard to blood pressure — both systolic and diastolic. The figures for the blood pressure are only given here as in the other tables in order to ascertain how large the differences were in this respect in the present material. In the other tests the figures were consistently lower for men in the group with blood pressure  $\geq 190$  mm Hg, with the exception of urea nitrogen where the figure was higher. There seem to be no such differences in the female group. For both the sexes together there was the same tendency

Table XXXIV. Mean (M) and standard error of the mean<sup>1</sup>( $\epsilon$ ) of different tests in cases with chronic nephritis distributed according to systolic blood pressure above and below 190 mm Hg.

Determination	Systolic blood pressure, mm Hg			
	$\geq 190$ mm Hg		< 190 mm Hg	
	Num- ber	M $\pm$ $\epsilon$ (M)	Num- ber	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance .....	10	36.1 $\pm$ (10.1)	15	48.8
Diodrast clearance .....	10	146.0 $\pm$ (46.2)	15	191.7
Filtration fraction .....	10	0.275 $\pm$ (0.031)	15	0.266
Creatinine clearance .....	5	28.4	2	81.0
Urea clearance .....	7	30.3	12	38.5 $\pm$ (4.4)
Effect. renal blood flow ....	10	236.7 $\pm$ (72.2)	15	328.9
Hæmatocrit .....	10	37.1 $\pm$ (2.1)	15	38.6
Proteinuria .....	10	0.99 $\pm$ (0.44)	15	1.99
Blood pressure { systolic ....	10	204.5 $\pm$ (9.6)	15	157.3 $\pm$ 5.2
	10	126.5 $\pm$ (6.6)	15	97.1 $\pm$ 4.4
Urea nitrogen, mg % .....	7	42.6	13	28.7 $\pm$ (4.7)
Women				
Inulin clearance .....	8	51.1	15	41.6 $\pm$ 6.0
Diodrast clearance .....	8	175.3	15	170.8 $\pm$ 29.0
Filtration fraction .....	8	0.288	15	0.277 $\pm$ 0.022
Creatinine clearance .....	2	49.0	6	59.8
Urea clearance .....	5	59.6	7	29.1
Effect. renal blood flow ....	8	277.5	15	265.6 $\pm$ 49.0
Hæmatocrit .....	8	37.8	15	32.7 $\pm$ 1.8
Proteinuria .....	8	3.97	15	1.72 $\pm$ 0.70
Blood pressure { systolic ....	8	206.9	15	155.7 $\pm$ 5.8
	8	125.6	15	97.3 $\pm$ 3.9
Urea nitrogen, mg % .....	7	27.0	9	33.7
Both sexes				
Inulin clearance .....	18	42.8 $\pm$ 7.9	30	45.2
Diodrast clearance .....	18	159.0 $\pm$ 28.6	30	181.3
Filtration fraction .....	18	0.281 $\pm$ 0.022	30	0.271
Creatinine clearance .....	7	34.3	8	65.1
Urea clearance .....	12	42.5 $\pm$ (7.3)	19	35.1
Effect. renal blood flow ....	18	254.8 $\pm$ 43.9	30	297.2
Hæmatocrit .....	18	37.4 $\pm$ 1.6	30	35.7
Proteinuria .....	18	2.31 $\pm$ 0.96	30	1.85
Blood pressure { systolic ....	18	205.6 $\pm$ 6.4	30	156.5 $\pm$ 3.8
	18	126.1 $\pm$ 5.1	30	97.2 $\pm$ 2.9
Urea nitrogen, mg % .....	14	34.8 $\pm$ (5.2)	22	30.8

<sup>1</sup> v. foot note p. 129

to differences as in the male group although this was less evident. The standard errors of the means were not, however, calculated in those with systolic blood pressure < 190 mm Hg, since it was obvious that no differences were even probable.

### The Diastolic Blood Pressure

The writer fixed 100 mm Hg as the borderline figure between the two sub-groups to be compared. The reason for this choice was the same as in the case of the systolic blood pressure, although the figure here may appear to be on the low side. Had a higher figure been chosen, the distribution in the two sub-groups would, however, have been still more unequal. Reference is made to fig 10 for a more detailed valuation of the level of diastolic pressure and its relation to the actual renal function.

Since the sub-groups were not sufficiently large in either sex to allow a comparison between them, calculations were made in this case as well for both sexes together (Table XXXV). The standard errors of the means were only calculated when it was evident that a possibility of a significant difference was present but none could be demonstrated. Almost throughout, however, signs of more impaired function in the groups with higher diastolic pressure seem to exist in all the tests both for men and women separately and for both sexes together.

### Non Protein Nitrogen or Urea Nitrogen

As a basis for the classification of the cases in this group the writer used the upper normal values for non protein nitrogen and urea nitrogen respectively, as given in Chapter I (p. 17). Difficulties occurred in placing a number of cases, i.e. those who for example had values under 40 mg per cent in one or two determinations of non protein nitrogen, but had a concentration of urea nitrogen that, according to the basis for this classification, was slightly raised. The following

Table XXXV. Mean (M) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) in cases with chronic nephritis distributed according to diastolic blood pressure.

Determination	Diastolic blood pressure, mm Hg			
	$\geq 100$		< 100	
	Number	M $\pm$ $\epsilon$ (M)	Number	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance . . . . .	16	38.4 $\pm$ 6.7	9	53.1
Diodrast clearance . . . . .	16	159.0 $\pm$ 30.9	9	199.1
Filtration fraction . . . . .	16	0.268 $\pm$ 0.022	9	0.273
Creatinine clearance . . . . .	6	41.5	1	55.0
Urea clearance . . . . .	13	33.8 $\pm$ (4.4)	6	39.2
Effect. renal blood flow . . .	16	273.1 $\pm$ 51.3	9	325.8
Hæmatocrit . . . . .	16	39.6 $\pm$ 1.8	9	35.2
Proteinuria . . . . .	16	1.78 $\pm$ 0.77	9	1.24
Blood pressure { systolic . . .	16	190.0 $\pm$ 8.2	9	151.7
	16	121.6 $\pm$ 4.9	9	86.2
Urea nitrogen, mg % . . . . .	13	37.8 $\pm$ (6.5)	7	25.6
Women				
Inulin clearance . . . . .	16	43.8 $\pm$ 7.4	7	47.6
Diodrast clearance . . . . .	16	159.3 $\pm$ 25.1	7	202.3
Filtration fraction . . . . .	16	0.290 $\pm$ 0.023	7	0.259
Creatinine clearance . . . . .	8	57.1	0	—
Urea clearance . . . . .	10	46.0 $\pm$ (7.9)	2	21.0
Effect. renal blood flow . . .	16	261.4 $\pm$ 43.0	7	288.7
Hæmatocrit . . . . .	16	36.9 $\pm$ 1.6	7	29.0
Proteinuria . . . . .	16	2.52 $\pm$ 0.99	7	2.47
Blood pressure { systolic . . .	16	181.9 $\pm$ 8.1	7	154.3
	16	116.9 $\pm$ 4.9	7	85.0
Urea nitrogen, mg % . . . . .	12	30.3 $\pm$ 3.6	4	32.2
Both sexes				
Inulin clearance . . . . .	32	41.1 $\pm$ 5.0	16	50.7
Diodrast clearance . . . . .	32	159.1 $\pm$ 19.6	16	200.5
Filtration fraction . . . . .	32	0.279 $\pm$ 0.016	16	0.267
Creatinine clearance . . . . .	14	50.4 $\pm$ (9.5)	1	55.0
Urea clearance . . . . .	23	39.1 $\pm$ 4.3	8	34.6
Effect. renal blood flow . . .	32	267.2 $\pm$ 32.9	16	309.6
Hæmatocrit . . . . .	32	38.2 $\pm$ 1.2	16	32.5 $\pm$ 2.0
Proteinuria . . . . .	32	2.15 $\pm$ 0.62	16	1.78
Blood pressure { systolic . . .	32	185.9 $\pm$ 5.7	16	152.8 $\pm$ 5.8
	32	119.2 $\pm$ 3.4	16	85.7 $\pm$ 1.9
Urea nitrogen, mg % . . . . .	25	34.2 $\pm$ 3.8	11	28.0

<sup>1</sup> v. foot note p. 129



principle was therefore adopted. If the majority of determinations gave normal values the patient was classified as normal. If two determinations only were made, the patient was classified according to the results of that of the non protein nitrogen. The reason for this procedure was that determination of non protein nitrogen is made as a matter of routine at the University Hospital in Upsala, whereas urea nitrogen is only determined in certain cases.

The figures for the different tests in these two groups are seen in Table XXXVI. The very unequal distribution both for men and for women made a calculation impossible for either sex separately. In view, however, of the evident difference present, the results would certainly have been the same as in the calculation made for both sexes together, i.e. significant lower values for the inulin and diodrast clearances, for effective renal blood flow and, of course, higher for urea nitrogen. The figures for the blood pressure were practically of the same order of magnitude in both groups, and this is also true of the filtration fraction. The haematocrit figures were, however, consistently lower in the group with higher non protein nitrogen, although the difference was not even probable. When computing the standard errors of the means of the smaller group, the method described on p. 150 was used.

### Haemoglobin Level

It has long been known that a correlation exists between anaemia and renal insufficiency. Brown and Roth (1923) thus found anaemia in 137 out of 139 patients with pronounced renal insufficiency, only two showing normal blood counts. Ashe (1929) also found a definite correlation between the degree of renal insufficiency and the blood level and he was of the opinion that anaemia of various types is often found in different kinds of renal insufficiency. Van Slyke, McIntosh, Møller, Hannon and Johnston (1929) and Bruger and Mosenthal (1932) found a definite correlation between the urea clearance and anaemia.

Table XXXVI. Mean (M) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) of different tests in cases with chronic nephritis distributed according to non protein nitrogen above or below 40 mg %.

Determination	Non protein nitr. > 40 mg % or Urea nitr. > 20 mg %		Non protein nitr. $\leq$ 40 mg % or Urea nitr. $\leq$ 20 mg %	
	Num- ber	M $\pm$ $\epsilon$ (M)	Num- ber	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance .....	20	32.0 $\pm$ 3.9	6	77.0
Diodrast clearance .....	19	135.5 $\pm$ 18.1	6	293.5
Filtration fraction .....	19	0.268 $\pm$ 0.020	6	0.273
Creatinine clearance .....	8	40.4	0	—
Urea clearance .....	13	28.9 $\pm$ (3.9)	6	49.7
Effect. renal blood flow ....	19	222.4 $\pm$ 32.3	6	512.7
Hæmatocrit .....	20	35.6 $\pm$ 1.7	6	44.0
Proteinuria .....	20	2.26 $\pm$ 0.79	6	0.90
Blood pressure { systolic ....	20	182.0 $\pm$ 9.2	6	170.8
	20	112.5 $\pm$ 5.8	6	103.5
Urea nitrogen, mg % .. ...	14	39.8 $\pm$ (5.6)	6	19.0
Women				
Inulin clearance .....	17	34.2 $\pm$ 4.0	5	80.8
Diodrast clearance .....	17	145.5 $\pm$ 23.8	5	255.6
Filtration fraction .....	17	0.272 $\pm$ 0.021	5	0.320
Creatinine clearance .....	5	50.8	2	69.5
Urea clearance .....	10	34.0 $\pm$ (5.7)	2	81.0
Effect. renal blood flow ....	17	225.6 $\pm$ 38.6	5	403.0
Hæmatocrit .....	17	33.7 $\pm$ 2.0	5	36.0
Proteinuria .....	17	2.5 $\pm$ 1.0	5	2.9
Blood pressure { systolic ....	17	170.6 $\pm$ 9.1	5	188.0
	17	107.4 $\pm$ 6.2	5	106.0
Urea nitrogen, mg % .....	12	35.6 $\pm$ (2.9)	4	16.3
Both sexes				
Inulin clearance .....	37	33.0 $\pm$ 2.8	11	78.7 $\pm$ (5.8)
Diodrast clearance .....	36	140.3 $\pm$ 14.6	11	276.3 $\pm$ (30.1)
Filtration fraction .....	36	0.270 $\pm$ 0.014	11	0.295
Creatinine clearance .....	13	44.4 $\pm$ (10.1)	2	69.5
Urea clearance .....	23	31.1 $\pm$ 3.3	8	57.5
Effect. renal blood flow ....	36	223.9 $\pm$ 24.6	11	462.8 $\pm$ (44.5)
Hæmatocrit .....	37	34.7 $\pm$ 1.3	11	40.4 $\pm$ (2.3)
Proteinuria .....	37	2.37 $\pm$ 0.62	11	1.81
Blood pressure { systolic ....	37	176.8 $\pm$ 6.5	11	178.6
	37	110.1 $\pm$ 4.2	11	104.6
Urea nitrogen, mg % .....	26	37.9 $\pm$ 3.3	10	17.9 $\pm$ (5.0)

<sup>1</sup> v. foot note p. 129

so that, for example, all cases with a haemoglobin level below 65 per cent (determined by Haldane's method) showed a urea clearance below 40 per cent. Since, however, it is considered that the urea clearance falls more rapidly than the haemoglobin level, it is possible to find cases of chronic nephritis with a haemoglobin level above 80 per cent but a urea clearance below 10 per cent. Parsons and Ekola-Strolberg (1933) summarized their own opinion on this matter and that of earlier workers in the following points:

1. Anaemia is almost consistently found in cases of azolaemia independently of the pathological background of the renal insufficiency.

2. In such cases anaemia is probably caused by decreased activity of the haemopoietic system.

3. The specific aetiological factor in such anaemia — if there is only one such factor — is unknown.

In consideration of the forementioned statements and others in the literature, the present writer wished to study the question of anaemia and renal function in his own material. Certain difficulties arose in fixing a borderline figure since the material was composed of both men and women. In order

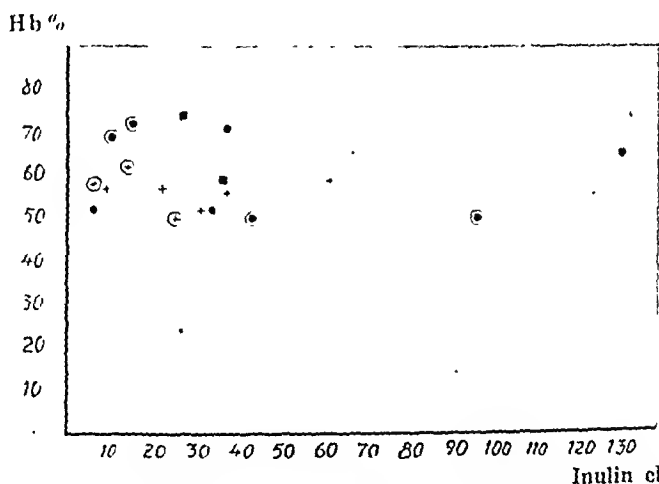


Fig 9. Diagram showing the haemoglobin in % (abscissa) in relation to inulin clearance in ml/min (ordinate). The small crosses indicate the cases of hyperchromic anaemia, the single dots those of hypochromic anaemia and the squares the normochromic cases. The deceased patients are designated by a ring round the symbol.

to ensure that no individuals with normal haemoglobin levels would be included in the anaemic group, the limit between the two sub-groups was therefore fixed at 75 per cent haemoglobin (Autenrieth's colorimetric method). It is obvious that different borderline figures should have been used for men and women and that several limits could have been used. The material was, however, too small to permit any more thorough investigation and the forementioned borderline figure was therefore chosen. Moreover, a division according to the different types of anaemia — hyper-, normo- or hypochromic — was not possible as the anaemic group was too small. Fig 9, however, gives a graphical survey of the distribution of these types of anaemia within the anaemic group in relation to the inulin clearance.

The results of the tests performed in both groups are seen from the survey in Table XXXVII. In the statistical calculation of the standard errors of the means, the same principle was applied as in the earlier classifications, i.e. a calculation was only made when the possibility of a difference was obvious. It is seen that the difference was statistically significant between the sub-groups only in the obvious case of the haematocrit determination. The filtration fraction and the figures for blood pressure were approximately on the same level in both groups. The other tests showed the same tendency throughout, with signs of more severely damaged renal function in the anaemic group, noticeable in both sexes. In view of the low filtration rate, it must be pointed out that «anaemia» may be caused simply by dilution of the blood. Hypochromic or hyperchromic anaemia cannot, however, be caused by this factor. It can therefore be of interest to emphasize that there were eight cases of hypochromic and eight of hyperchromic anaemia. Thus there were only two cases with a normal haemoglobin content in the red blood corpuscles which could be thought to be connected with a dilution of the blood. If the disturbance in this respect had been more severe, oedema could have been expected in the patients in question. Only one patient, however, manifested this symptom.

Table XXXVII. Mean (M) and standard error of the mean<sup>1</sup> (s) in cases with chronic nephritis distributed according to haemoglobin values.

Determination	Haemoglobin value			
	< 75 %		≥ 75 %	
	Num- ber	M ± s(M)	Num- ber	M ± s(M)
Men				
Inulin clearance .....	7	20.0	18	52.1 ± 6.7
Diodrast clearance .....	6	100.2	18	199.2 ± 28.7
Filtration fraction .....	6	0.247	18	0.283 ± 0.018
Creatinine clearance .....	3	25.7	5	49.2
Urea clearance .....	3	12.7	16	39.8 ± 3.7
Effect. renal blood flow ....	6	142.7	18	346.8 ± 47.9
Hæmatocrit .....	7	28.2	18	41.7 ± 4.4
Proteinuria .....	7	2.71	18	1.74 ± 0.69
Blood pressure { systolic ....	7	197.1	18	174.7 ± 6.6
	7	112.1	18	111.2 ± 5.0
Urea nitrogen, mg % .....	3	53.8	17	30.0 ± 3.9
Women				
Inulin clearance .....	11	45.2 ± (11.4)	10	47.4
Diodrast clearance .....	11	170.5 ± (36.1)	10	185.4
Filtration fraction .....	11	0.282 ± (0.029)	10	0.272
Creatinine clearance .....	3	32.0	5	72.2
Urea clearance .....	6	34.8	5	52.6
Effect. renal blood flow ....	11	256.7 ± (56.5)	10	309.0
Hæmatocrit .....	11	32.4 ± (1.5)	10	39.6 ± (1.6)
Proteinuria .....	11	2.4 ± (1.1)	10	2.9
Blood pressure { systolic ....	11	169.1 ± (6.7)	10	182.0
	11	102.3 ± (4.5)	10	117.0 ± (8.4)
Urea nitrogen, mg % .....	8	34.4	7	26.9
Both sexes				
Inulin clearance .....	18	35.4 ± 7.6	28	50.4
Diodrast clearance .....	17	145.6 ± 26.5	28	194.3
Filtration fraction .....	17	0.269 ± 0.022	28	0.279
Creatinine clearance .....	6	28.8	10	60.7 ± (16.8)
Urea clearance .....	9	27.4	21	42.8 ± 3.5
Effect. renal blood flow ....	17	216.4 ± 41.1	28	333.3 ± 34.9
Hæmatocrit .....	18	30.8 ± 1.1	28	41.0 ± 2.9
Proteinuria .....	18	2.50 ± 0.84	28	2.14
Blood pressure { systolic ....	18	180.0 ± 9.0	28	177.3
	18	106.1 ± 5.5	28	113.3
Urea nitrogen, mg % .....	11	39.7 ± (6.6)	24	29.1

<sup>1</sup> v. foot note p. 129

### *Summary and Discussion*

To sum up the results it can be said that all the tests indicate impaired renal function in several respects in this disease as compared with individuals with healthy kidneys.

As regards the facultative symptoms (raised blood pressure and non protein nitrogen, retinal changes and anaemia) they occurred in 33-77 per cent of the cases (v. Table XXXI, p. 147).

In a calculation of expected and observed values in pairs of different tests outside certain limits for normal values no correlation could be demonstrated, possibly due to the small material.

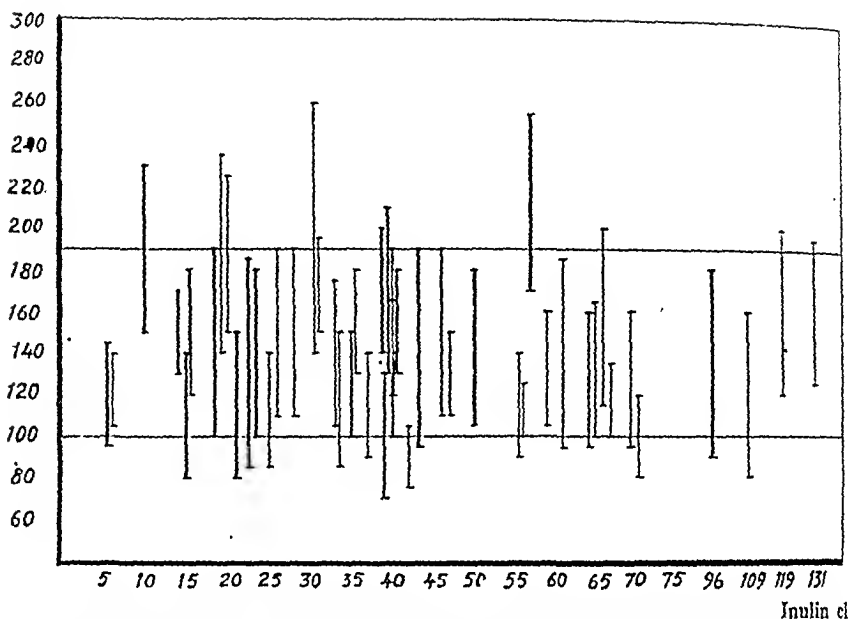
By grouping the material according to the degree of various symptoms it is moreover possible to state the following. In chronic nephritis with pronounced *retinal changes*, corresponding to Keith's groups III and IV, there seems to be a tendency to lower values in this group compared with those without these changes as regards inulin and diodrast clearances and significant higher values in respect of the systolic and diastolic blood pressure and the concentration of urea nitrogen in the plasma.

The same tendency is found in the sub-divisions in the majority of the other groupings, i.e. lower figures for renal function and parallel to them higher figures for urea nitrogen concentration when the systolic blood pressure was  $\geq 190$  mm Hg, the diastolic blood pressure  $> 100$  mm Hg and the haemoglobin level  $\leq 75$  per cent (Anthenrieth). Within all these groupings the difference between the sub-groups is only significant in the case of that figure on which the classification was based.

In classification according to normal or raised *non protein nitrogen*, on the other hand, significant lower values are found in the latter sub-group for both sexes for the inulin and diodrast clearances and the effective renal blood flow, i.e. differences for such factors that more or less directly cause the retention of nitrogenous substances.

The question of the degree of elevation of the blood pressure and its correlation to renal function has already been touched

B P



*Fig 10.* Diagram showing the blood pressure (abscissa) at different inulin clearances (ordinate). The thick lines mark the blood pressure of patients still alive. The top of the lines marks the systolic blood pressure, the base the diastolic pressure. The upper horizontal thin line indicates the borderline of the systolic blood pressure in this grouping and the lower that of the diastolic pressure.

on. Fig 10 shows the general tendency to raised systolic — and particularly diastolic — blood pressure with increasing renal impairment. It is also seen from this figure that the amplitude of the pulse was approximately the same at high or low renal function figures both in patients who died within 11 months and those who survived. It must be pointed out that in accordance with the requirements for life insurance policies in Sweden, the diastolic blood pressure was registered when the arterial sound was no longer heard.

The significance of anaemia in the actual state of renal function has also been mentioned earlier. Fig 9 nevertheless brings two further facts to light: 1. anaemia, at any rate in the present material, was not particularly severe; 2. in about 56 per cent of the anaemic cases it was of normo- or hyperchromic type. That it occurs particularly in patients with

severely impaired renal function is also entirely in agreement with earlier investigations.

As the survey of the literature at the beginning of the present chapter shows, some workers have emphasized that the filtration fraction is high in advanced cases of chronic nephritis (Earle, Taggart and Shannon 1944). Hilden (1946) came to the same conclusion in his investigation of 23 cases of chronic nephritis, although he determined the quotient of  $\frac{\text{urea clearance} \times 100}{\text{diodrast clearance}}$  instead of the filtration fraction.

Eckhorn (1946) was of the opinion, however, that in decreasing renal function the urea rediffusion becomes increasingly large and it therefore follows that the urea clearance becomes proportionately increasingly low in relation to the actual volume of the filtrate. Were this true, and provided that inulin does not rediffuse in a severely impaired kidney — and there is no *definite* proof that this does occur — Hilden's figures must represent minimum figures. It must, however, be assumed in Hilden's severe cases that the quantity of diodrast injected was considerably less than in the normal cases, since otherwise the low diodrast clearance in the former could be caused to a considerable extent by a too high concentration of diodrast in the plasma (v. p. 44).

The present writer's determinations of the filtration fraction are depicted graphically in fig. 11. The distribution on either side of the normal figure is approximately the same and it is therefore not surprising that the mean figure was the same as for persons with healthy kidneys. On closer study of this graph it is nevertheless seen that at a filtration rate below 50-60 ml/per minute, the prognosis *quoad vitam* is considerably poorer for the cases with a high filtration fraction than for those with a low. Four out of 28 patients died in the latter group of which one (filtration 97 ml/min) 2½ years after the investigation, one (43 ml/min) — the only instance in the material — of cerebral haemorrhage 4 months after the investigation and the other two (filtration 10 ml/min or lower) were at an advanced uraemie



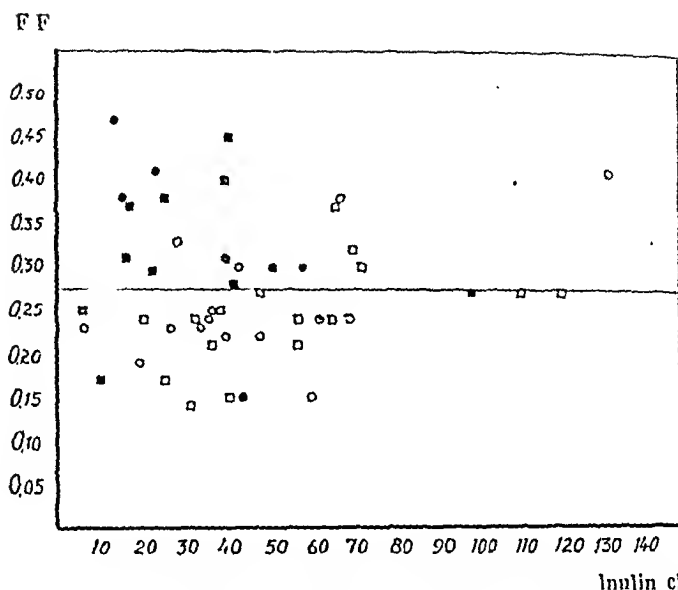


Fig 11. Diagram showing the filtration fraction at different inulin clearances. The squares indicate men, circles the women. Filled symbols denote patients who have died. The horizontal line marks the normal value for the filtration fraction.

stage at the time of the investigation. In the group with high filtration fractions all the deaths occurred within one to eleven months after the investigation, with the exception of three cases. In one of them — the only one of its kind — the outcome was fatal only after 3 1/2 years, despite the fact that during this time repeated tests showed an inulin clearance of 16 ml per minute or lower and, naturally, a considerable increase in non protein nitrogen (80-170 mg per cent).

In the present writer's opinion, the fact that an increase in the mortality is found in patients with very low filtration and a high filtration fraction is a strong proof of the value of determinations of the inulin and diodrast clearances as a prognostic guide in chronic nephritis.

The following fact gives further support to this theory. In the whole material there were five cases with very low filtration rates in which the diagnosis of chronic nephritis was fully justified but in which continued observation with clearance determinations repeatedly showed that it could not have been progressive chronic nephritis but probably an acute

exacerbation of incompletely healed acute nephritis. In all these cases the filtration fraction was below normal thus indicating a better prognosis.

The present material is too small and the observation time in a number of cases too short to prove beyond doubt the writer's conclusion. Nevertheless, the tendency in this material is very suggestive.

As regards the prognosis in general, it is seen from Table XXXII, in which the material is grouped according to the various clinical grounds for classification, that the mortality rate is highest in those groups in which it could be expected to be high in the light of our present knowledge. The results of the functional tests confirm these conclusions, i.e. the greater the impairment of renal function the poorer is the prognosis.

One final remark is warranted before concluding this chapter. Chronic nephritis is usually divided into three stages:

1. the latent stage.
2. the chronic active stage. and
3. the final stage.

There is usually no difficulty in making either a diagnosis or a prognosis in the third stage, but it is hardly possible at a single examination to differentiate between the first two groups. The object of the present investigation has been, *inter alia*, to make some small contribution to the solution of this problem.

## CHAPTER VI.

# ESSENTIAL HYPERTENSION

### Earlier Investigations

That the blood pressure is elevated in renal disease has been known since Sanderson pointed out this fact about 1868. Already in 1896, however, Allbutt emphasized that elevated blood pressure could exist as a specific disease without connexion with Bright's disease, and he named this condition *hyperpiesia*. The present name of the disease, i.e. essential hypertension, dates from Frank (1911).

It is impossible in the present paper to make a closer survey of all the investigations carried out to elucidate the pathogenesis of this condition. Reference is therefore made to recent monographs on the subject (e.g. Bechgaard 1946). It must, however, be emphasized that the pathogenesis is not uniform and several classifications of hypertensive disease have therefore been proposed. The best-known are those of Volhard (1923), Schroeder and Steele (1939), Böger and Wezler (1939) and Ask-Upmark (1942).

It has been mentioned that elevated blood pressure was formerly assumed to be associated with renal disease. With increased knowledge of the subject and possibly chiefly on the basis of the work carried out by Volhard and his collaborators, the opinion became accepted that the hypertensive disease *sui generis* was not associated with renal disease except in severe cases when the kidneys could secondarily become the site of lesions. Nevertheless, through Hartwich's (1930) and particularly Goldblatt's (1934) pioneer work on experimental hypertension, attention was once again focused on the kidney. Extensive experiments

have been carried out in order to demonstrate hypertensive substances in the kidney, on the basis of the enzymatic protein, renin, demonstrated by Tigerstedt and Bergmann in 1898, (Houssay, Braunn-Menendez et al., Page et al., von Euler and Sjöstrand, and others).

The optimism aroused in the beginning of what can be termed the era of experimental hypertension has now possibly been superseded by a pessimistic outlook regarding the possibility of solving the mystery of hypertension along these lines. The present writer performed clearance determinations in a number of such patients since the relatively few large investigations of this kind of which reports have been published partly contradict each other. Moreover, when the present investigation was started in 1912, no extensive investigation was known to the present writer.

Before a short summary of earlier such investigations is given, some mention must be made of the terminology of this disease. Volhard and Fahr (1911) classified hypertension as: 1. benignant hypertension and 2. malignant nephrosclerosis. They based their classification on the clinical observation that the prognosis is good in some forms of hypertension where the patients have no severe renal symptoms, whereas the prognosis is poor in other cases, when the disease has a rapid course usually with retinopathy and with pronounced renal lesions, manifest as more or less marked pathological findings in the urine. This classification is of practical use and has therefore also been used by other workers, even if the names given to the two groups vary. Since retinal changes are easily registered and are nearly always decisive for the prognosis, Wagener and Keith (1939), divided hypertensive disease into four groups (v. p. 180), of which their group IV belongs to Volhard and Fahr's group termed malignant nephrosclerosis and their group III is in an intermediate position to groups 0-II, which are included in the benignant group. The best basis for classification seems to be to differentiate only between benignant and malignant hypertension. To the former group belong those cases of hypertension that are not conditioned

by renal disease or caused by endocrine disturbances, coarctation of the aorta or other well-known factors and in which the condition implies relatively little danger to the kidneys. To the latter group belong such cases that have a poor prognosis. Pronounced retinopathy is then usually present as well as constantly pathological urine as a sign of more or less severe involvement of the kidneys, and uraemia is often the cause of death. The term nephrosclerosis should perhaps not be used, since it implies an aetiological knowledge and is therefore frequently misleading. Whether the term »malignant hypertension» or »the malignant phase of essential hypertension» is used — the latter being Fishberg's (1939) term — is to a certain extent a matter of taste, since the question of whether we are dealing with various types of diseases or different stages of the same disease will not be discussed here.

Lundsgaard and Møller (1925) found that in the majority of cases investigated there was a decreased figure for the phenolsulphonphthalein test according to Rowntree and Geraghty and they considered that this was due to a decreased renal blood flow. Neubauer (1914) found a decreased excretion of creatinine in some hypertensive patients compared with healthy individuals after a tolerance test with 1.5 g of creatinine. Major (1928) found, after intravenous injection of 0.25 g of creatinine, a decreased excretion of this substance in approximately 35 per cent of hypertensives. Høllen and Rehberg (1929) found normal filtration, determined by creatinine clearance, in four out of five hypertensives and a slight decrease in the remaining patient. Data concerning the degree of hypertension and whether it was benignant or malignant or both are, nevertheless, lacking in the reports of all the forementioned writers. Roelsen (1932), who also determined the creatinine clearance in 25 patients with essential hypertension, found normal filtration in 17 and a decrease in the remaining eight cases.

Ellis and Weiss (1933) determined the urea and creatinine clearances and the maximal concentration capacity in 24 hypertensives. Ten of them showed normal urea clear-

ances and 13 normal creatinine clearances. In 22 concentration tests, six patients had figures over 1.025, ten had figures between 1.020 and 1.025 and six had figures below 1.020. The writers found a certain parallelism between the blood pressure — particularly the diastolic — and renal function: the higher the blood pressure the poorer the function. On the other hand, they stated that there was no parallelism between the age of the patient or the duration of the symptoms and the functional changes.

Smith, Goldring, Chasis and Ranges (1938), who examined patients with hypertension and made clearance determinations with inulin and diodrast, found that these patients consistently showed a lower renal blood flow than normal patients or those with chronic nephritis and equally pronounced renal lesions. They interpreted this as a sign that certain tubules had lost their power of excretion but had maintained their connexion with the respective glomeruli and that they could therefore serve as passive conductors for the glomerular filtrate, with the result that the volume of the filtrate was excessive in relation to the number of residual functioning tubules. The same group of workers published in 1940 a collation of clearance tests in 60 hypertensive patients. For the whole group the diodrast  $T_m$  was low or at the lower level of the normal figures. This was assumed to indicate that the disease is characterized by a progressive impairment of tubular function. It was found that in some individuals the tubular lesion was greater than the impairment of glomerular function. On the basis of their earlier assumptions the forementioned writers interpreted this as the formation of inactive tubules. In other cases they found that the effective blood flow per unit of functional tubular tissue (diodrast clearance/diodrast  $T_m$ ) was decreased in comparison with normal individuals, indicating relative renal ischaemia. Since this ischaemia is accompanied by an increase in the filtration fraction it is probably connected with an increased tonus in the efferent glomerular arterioles. This increased tonus is in turn functionally reversible in some cases but not in others.

Chesley and Chesley (1940) made an investigation on 37 female patients with hypertension. The majority had an evident decrease in renal blood flow, some had a slight decrease and in a few cases it was normal. The filtration fraction was high, thus explaining that it is possible to obtain »normal» renal function with other tests than diodrast clearance. Chasis and Redish (1941 and 1942) also found a decrease in the renal blood flow, measured by diodrast clearance, in 22 hypertensive patients. They also emphasized that renal ischaemia is not necessarily manifested by a decreased blood flow only, but actually by a decrease in this blood flow per unit of tubular excretory tissue. Determination of the diodrast clearance and the diodrast  $T_m$  gave no grounds for assuming the presence of unilateral renal ischaemia; the reduction in these functions was equally distributed in both kidneys.

Foa, Woods, Peet and Foa (1942) investigated 20 hypertensive patients and came to the same conclusion as earlier workers, i.e. there is a reduced effective renal blood flow, probably dependent on increased tones in the efferent arterioles, and a raised filtration fraction. Steinitz (1941) also used the inulin and diodrast clearances in his investigation on six cases of essential hypertension (benignant hypertension) and three cases of malignant hypertension. In the former group the inulin clearance was somewhat lower than normal throughout and the diodrast clearance proportionately lower, the filtration fraction thus being higher than normal. According to Steinitz, this depended on the fact that two of the six cases had pronounced changes of this kind in renal function and their figures thus influenced those of the group as a whole. He therefore concluded that, with a normal filtration rate, a decrease in renal blood flow is not necessarily an indication of essential hypertension and that thus, in the majority of cases, the kidney is not involved in a kind of vicious circle. In all three cases with malignant hypertension he found decreased filtration, a proportionately lower diodrast clearance and thus a higher filtration fraction than in the normal group and in those with essential (be-

nignant hypertension. Findley, Edwards, Clinton and White (1942) reached the following conclusions based on the results of their study of twelve patients with essential hypertension, all under the age of 50, with normal urea clearance and no pathological sediment, no retinal changes or cardiac involvement, etc. »1). The plasma clearance of diodrast and inulin, even when interpreted in the light of tubular secretion of diodrast, indicates the absence of renal ischaemia in a high proportion of subjects with uncomplicated essential hypertension. 2) Under controlled conditions the ratio between inulin clearance and diodrast clearance can represent the 'filtration fraction', but high ratios in subjects with hypertension will probably result from diminished diodrast extraction rather than from increased filtration pressure».

A statistical analysis of 100 patients with essential hypertension compared with 44 normal individuals was made by Dalton and Nuzum (1942) on the grounds that in a comparison between individuals there is no changes in renal function measured with the concentration and phenolsulphonphthalein tests, but that in comparing whole groups the following statements can be made:

1. In essential hypertension the age of the patient or the duration of the disease has no influence on renal function.
2. A retardation of the flow of urine occurs with rising diastolic blood pressure.
3. In hypertensive patients there is usually a fixation of the specific gravity of the urine and a retardation of phenolsulphonphthalein output.

It is difficult to judge how far these conclusions are justified by their material which is rather small from a statistical point of view.

According to the forementioned writers, neither Volhard's concentration test nor the phenolsulphonphthalein test are of any great value for the differential diagnosis between normal subjects and patients with essential hypertension in individual cases.

Goldring and Chasis (1944) summarize the results



of their study of 60 cases with essential hypertension as follows: Hypertensive disease in man is associated with progressive changes in renal function and renal haemodynamics. In the majority of patients this process is slow, in others it is rapid. At an early stage of hypertensive disease there is a decrease in the excretory power of the tubules associated with a reduction in the effective renal blood flow. The filtration, on the other hand, is normal in the early stage and the filtration fraction high. These three symptoms, i.e. decreased tubular secretion, decreased effective renal blood flow and a high filtration fraction are present in practically every patient with well-established hypertension. When the renal disease progresses, the inulin and urea clearances also gradually begin to decrease, although proportionately less than the diodrast clearance. The maximal concentrating power and the phenolsulphonphthalein excretion are, however, normal or only slightly decreased for a comparatively long period in the beginning. Only in the final stage do these functions show reduced figures.

Hilden (1946) also made an investigation on 60 cases of hypertension by means of diodrast and urea clearances. He found in a number of patients with benignant hypertension that both clearances were normal as well as the U/D quotient (urea clearance/diodrast clearance). In others, both clearances were lowered. In malignant cases of hypertension he found that the diodrast clearance was nearly always definitely decreased but the urea clearance could be normal, slightly or exceedingly decreased and the quotient of U to D therefore usually raised. In agreement with other writers, he considered the cause to be an increased tonus in the efferent arterioles.

### Tests in Cases with Hypertension

The present material consisted of 62 patients with essential hypertension, 20 men and 42 women. The distribution according to age is seen in Table XXXVIII, in which the number of deaths is also given. These cases were not selected, with the exception that milder cases were under-represented. This is

Table XXXVIII. Cases of essential hypertension distributed according to age and symptoms of different kinds. Figures inside brackets apply to cases dead within 3 years (they are included in the figures without brackets).

Age, years	All cases	Retinal changes		Syst. blood pressure mm Hg		Diast. blood pressure mm Hg		Non prot. nitrogen mg %		Duration			
		0 — II	III — IV	≥ 210	< 210	≥ 120	< 120	> 40	≤ 40	> 3 years	< 3 years		
Men													
30	1	A <sub>1</sub>	A <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	C <sub>1</sub>	C <sub>2</sub>	D <sub>1</sub>	D <sub>2</sub>	E <sub>1</sub>	E <sub>2</sub>		
31 — 40	3 (1)	1	—	—	1	—	1	—	1	1	—	—	
41 — 50	3	2	1 (1)	2 (1)	1	2 (1)	1	2 (1)	1	2 (1)	1	—	
51 — 60	8 (4)	3	—	1	2	1	2	—	3	1	1	—	
61 — 70	4	5 (2)	3 (2)	5 (3)	3 (1)	5 (2)	3 (2)	5 (3)	3 (1)	5 (2)	3 (2)	—	
> 71	1 (1)	3	1	2	2	2	2	2	2	2	3	—	
Total	20 (6)	15 (3)	5 (3)	10 (4)	10 (2)	10 (3)	10 (3)	10 (5)	10 (1)	11 (3)	9 (3)	—	
Women													
30	0	—	—	—	—	—	—	—	—	—	—	—	
31 — 40	5	5	—	2	3	4	1	1	4	1	4	—	
41 — 50	7 (1)	6	1 (1)	6 (1)	1	5 (1)	2	2 (1)	5	2 (1)	5	—	
51 — 60	14 (2)	12 (1)	2 (1)	8 (2)	6	8 (2)	6	6 (1)	8 (1)	4 (2)	10	—	
61 — 70	11 (3)	9 (1)	2 (2)	7 (3)	4	6 (3)	5	7 (2)	4 (1)	5 (2)	6 (1)	—	
> 71	5 (2)	4 (1)	1 (1)	4 (2)	1	3 (2)	2	2 (2)	2	4 (2)	—	—	
Total	42 (8)	36 (3)	6 (5)	27 (8)	15	26 (8)	16	18 (6)	23 (2)	16 (7)	25 (1)	—	
Total no.	62	51	11	37	25	36	26	28	33	27	34	—	
Dead	14	6	8	12	2	11	3	11	3	10	4	—	
% deaths	22.6	11.8	72.8	32.4	8.0	30.6	11.5	39.3	9.1	37.0	11.8	—	

owing to the fact that mild cases that were hospitalized usually showed a decrease in blood pressure after a short stay in hospital — usually owing to some other illness — as an expression of the instability of the blood pressure in the beginning of the hypertensive disease. Since they then showed no elevation of the blood pressure they were not included in the material. Another reason for the limited number of early and mild cases is that the patient material was taken from the wards and not from the Out-Patients' Department in which such patients are most frequently treated. Nevertheless, the cases included in this group were thoroughly examined with regard to the occurrence of symptomatic hypertension. The diagnosis of »essential hypertension» could only be made on the basis of the clinical symptoms. A diagnosis of this kind should, however, be fairly certain. The patients in this group can thus to some extent be said to represent selected cases, but on the other hand the diagnosis was confirmed by means of all the methods of examination available.

Table XXXIX gives the figures for the various tests. No difference between men and women could be anticipated and no calculation was therefore made of the possible differences between the sexes. Moreover, such a calculation would have been pointless since, from some aspects, it was impossible to compare the two groups. Table XXXX gives the differences between the means of this group and of the normal individuals. It is seen that the differences were statistically significant in all the tests if the calculations were made for both sexes together, with the exception of the urea nitrogen in the plasma, for which the difference was only approximately twice the standard error. The difference in the haematocrit figure was significant neither for the men nor for the women, but a calculation for both sexes together gave a statistically probable difference ( $>2.5$  times the standard error). It must be particularly emphasized that the figure for the filtration fraction was significantly higher both for men and for women in comparison with the normal material and thus naturally also for both sexes together. This fact is.

Table XXXIX

Mean (M), standard error of the mean ( $\epsilon$ ) and standard deviation ( $\sigma$ ) of different tests for men and women with essential hypertension.

Determination	Men			Women			Both sexes		
	Num- ber	M $\pm$ $\epsilon$ (M)	$\sigma$	Num- ber	M $\pm$ $\epsilon$ (M)	$\sigma$	Num- ber	M $\pm$ $\epsilon$ (M)	$\sigma$
Inulin clearance .....	20	91.1 $\pm$ 6.6	29.6	42	91.1 $\pm$ 3.3	21.2	62	91.1 $\pm$ 3.0	23.8
Diodrast clearance .....	20	262.3 $\pm$ 35.6	159.2	42	279.2 $\pm$ 12.2	79.3	62	280.2 $\pm$ 11.6	91.7
Filtration fraction .....	20	0.343 $\pm$ 0.020	0.089	42	0.339 $\pm$ 0.010	0.066	62	0.3400 $\pm$ 0.0092	0.0721
Creatinine clearance .....	5	104.4	—	13	99.6 $\pm$ (8.0)	—	18	100.9 $\pm$ 6.3	26.6
Urea clearance .....	10	55.2 $\pm$ (7.8)	—	32	61.7 $\pm$ 3.8	21.5	42	60.1 $\pm$ 3.4	22.2
Effect. renal blood flow ....	20	523.3 $\pm$ 50.4	225.5	42	495.1 $\pm$ 22.3	144.8	62	504.2 $\pm$ 21.9	172.1
Hæmatocrit .....	20	44.2 $\pm$ 1.7	7.5	42	43.31 $\pm$ 0.56	3.63	62	43.60 $\pm$ 0.65	5.10
Proteinuria .....	19	0.116 $\pm$ 0.055	0.239	42	0.114 $\pm$ 0.063	0.406	61	0.115 $\pm$ 0.046	0.358
Blood pressure { systolic .... diastolic....	20	198.8 $\pm$ 7.6	33.9	42	218.6 $\pm$ 5.8	37.6	42	218.6 $\pm$ 5.8	37.6
	20	119.5 $\pm$ 5.5	24.4	42	121.9 $\pm$ 2.9	18.9	42	121.9 $\pm$ 2.9	18.9
Urea nitrogen, mg % .....	15	24.7 $\pm$ 2.8	10.8	35	20.21 $\pm$ 0.81	4.79	35	20.21 $\pm$ 0.81	4.79

Table XXXX

Differences between normal cases and cases with essential hypertension with regard to different tests. A minus difference signifies that the normal figure is larger, a plus difference signifies the contrary.

Determination	Diff. between normal cases and cases with essential hypertension			
	Men	Women	Both sexes	
Inulin clearance . . . . .	$-33.0 \pm 7.0$	$-27.7 \pm 4.4$	$-31.1 \pm 3.5$	
Diodrast clearance . . . .	$-186.6 \pm 36.3$	$-157.2 \pm 15.8$	$-164.0 \pm 12.9$	
Filtration fraction . . . . .	$+0.066 \pm 0.021$	$+0.065 \pm 0.013$	$+0.065 \pm 0.010$	
Creatinine clearance . . .	$-12.6$	$-50.8 \pm (9.5)$	$-40.0 \pm 8.2$	
Urea clearance . . . . .	$-27.2 \pm (9.4)$	$-13.5 \pm 8.2$	$-20.2 \pm 5.2$	
Effect. renal blood flow .	$-329.6 \pm 51.6$	$-267.8 \pm 26.8$	$-314.7 \pm 24.3$	
Hæmatocrit . . . . .	$-3.18 \pm 1.74$	$-0.53 \pm 0.85$	$-2.13 \pm 0.81$	
Blood pressure {	systolic .	$+68.4 \pm 7.7$	$+88.1 \pm 6.0$	$+88.2 \pm 5.9$
	diastolic .	$+43.7 \pm 5.5$	$+44.9 \pm 3.2$	$+45.7 \pm 3.0$
Urea nitrogen, mg % ..	$+7.5 \pm 2.9$	$+1.42 \pm 1.62$	$+2.54 \pm 1.10$	

evident from the figures in Table XXXIX in which the filtration rate (inulin clearance) was proportionately less decreased in relation to that of the normal material than the diodrast clearance, in which the figure was only approximately 59 per cent of the latter, and the relation between them, i.e. the filtration fraction was thus higher than normal.

Table XXXXIII shows, as in the case of acute and chronic nephritis, how often the results of the various tests fall outside the normal limits of variation. It is found that 65 per cent lie outside the  $3\sigma$  limit — i.e. are definitely pathological — in respect of the diodrast clearance whereas the inulin clearance was decreased and the filtration fraction increased only in approximately 30 per cent. The percentage of cases with a decrease in urea clearance and increase in urea nitrogen is also lowest in this group (7-10 per cent). It is, however, striking that the number of cases with lowered creatinine clearance is so small (11 per cent) and considerably smaller than the number with lowered inulin clearance. This is probably due to the fact that creatinine clearance determinations

were only made on a small part of the material of which the majority suffered only from relatively slight hypertension.

In order to ascertain whether there was any correlation between the changes in two different tests, a calculation was made for this hypertensive material of the expected and observed figures in the same ways as for the two preceding groups of disease (Table XXXXI).

The greatest correlation appears here to exist between the inulin and creatinine clearances, which could be expected since both are to a great extent an expression of the size of the filtration rate. It is not surprising to find that there

Table XXXXI

Expected and observed values (in per cent) of cases with essential hypertension outside certain limits for normal values (2  $\sigma$  and 3  $\sigma$ ) in pairs of different tests<sup>1</sup>.

Determination		Number	Expected value, %		Observed value, %	
			2 $\sigma$	3 $\sigma$	2 $\sigma$	3 $\sigma$
Inulin clearance	— Diodrast clearance	62	47 $\pm$ 6.3	19 $\pm$ 5.0	55	29
Inulin clearance	— Filtration fraction ..	62	28 $\pm$ 5.7	10 $\pm$ 3.8	29	11
Inulin clearance	— Creatinine clearance	18	19 $\pm$ 9.2	3 $\pm$ 4.0	33	11
Inulin clearance	— Urea clearance ....	42	12 $\pm$ 5.0	2 $\pm$ 2.2	17	7
Inulin clearance	— Urea nitrogen, mg %	50	10 $\pm$ 4.2	3 $\pm$ 2.4	16	8
Diodrast clearance	— Filtration fraction ..	62	39 $\pm$ 6.2	22 $\pm$ 5.3	47	31
Diodrast clearance	— Creatinine clearance	18	27 $\pm$ 10.5	7 $\pm$ 6.0	33	11
Diodrast clearance	— Urea clearance ....	42	17 $\pm$ 5.8	5 $\pm$ 3.4	21	7
Diodrast clearance	— Urea nitrogen, mg %	50	15 $\pm$ 5.0	7 $\pm$ 3.6	18	10
Filtration fraction	— Creatinine clearance	18	16 $\pm$ 8.6	4 $\pm$ 4.6	22	6
Filtration fraction	— Urea clearance ....	42	10 $\pm$ 4.6	2 $\pm$ 2.2	12	2
Filtration fraction	— Urea nitrogen, mg %	50	9 $\pm$ 4.0	3 $\pm$ 2.4	8	6
Creatinine clearance	— Urea clearance ....	9	7	1	11	0
Creatinine clearance	— Urea nitrogen, mg %	9	6	1	11	0
Urea clearance	— Urea nitrogen, mg %	42	4 $\pm$ 3.0	1 $\pm$ 1.5	12	5

<sup>1</sup> The expected values are obtained simply as a product of the frequency of the respective tests (v. Table XXXXIII) and should apply if there was only a random combination.

Table XXXXII  
Grouping of some symptoms in cases with essential hypertension.

Symptom	Systolic blood pressure		Diastolic blood pressure		Retin. changes		Non protein nitrogen		Duration	
	$\geq 210$ mm Hg	$< 210$ mm Hg	$\geq 120$ mm Hg	$< 120$ mm Hg	0 - II	III - IV	$> 40$ mg %	$\leq 40$ mg %	$> 3$ years	$< 3$ years
No. of cases	36	26	35	27	51	11	28	33	27	34
Per cent	58 %	42 %	57 %	43 %	82 %	18 %	46 %	54 %	44 %	56 %

Table XXXXIII  
Number and percentage of individuals with essential hypertension falling outside the normal limits calculated according to  $2\sigma$  and  $3\sigma$  respectively.

Determination	No.	$2\sigma$	$3\sigma$
Inulin clearance	62	36 (58 %)	18 (29 %)
Diodrast clearance	62	50 (81 %)	40 (65 %)
Filtration fraction	62	30 (48 %)	21 (34 %)
Creatinine clearance	18	6 (33 %)	2 (11 %)
Urea clearance	42	9 (21 %)	3 (7 %)
Urea nitrogen, mg %	50	9 (18 %)	5 (10 %)

is also a mutual relation between the inulin and diodrast clearances. This would indicate that both the glomerular and tubular function is damaged. The correlation between the diodrast and creatinine clearances could perhaps have been expected to appear more sharply than it does since both tests — although to a different extent — are connected with tubular function. That the figures are not higher can depend on the fact that the number of cases in which both these tests were performed is small (only 18). In other words the chance of random variations is considerable. The table shows that a correlation also seems to exist between the diodrast clearance and the filtration fraction, the inulin and urea clearances and, obviously, between the last-mentioned and urea nitrogen.

The symptomatology of essential hypertension was touched on earlier in the account of the composition of the material (v. p. 97). In order to be able to survey the frequency of the objective symptoms in the present material more easily, a frequency table has been compiled (v. Table XXXXII) showing the blood pressure (systolic and diastolic), retinal changes (Keith's groups 0-IV) non protein nitrogen and also the duration of the disease in those cases in which it was possible to make a fairly accurate calculation.

In order to study the importance of various factors in the disturbance of renal function apparent from the study of the material as a group, the writer — as in the case of acute and chronic nephritis — divided the material into a number of sub-groups. The classifications were based on significant clinical data and the duration of the disease.

The sub-groups were therefore as follows:

- A. 1. Cases with retinal changes 0-II.
2. Cases with retinal changes III-IV.
- B. 1. Cases with systolic blood pressure  $\geq 210$  mm Hg.
2. Cases with systolic blood pressure  $< 210$  mm Hg.
- C. 1. Cases with diastolic blood pressure  $\geq 120$  mm Hg.
2. Cases with diastolic blood pressure  $< 120$  mm Hg.



for the inulin and diodrast clearances, and the effective renal blood flow were obtained in the group with pronounced retinal changes and higher values for the systolic blood pressure. The differences were statistically significant with the exception of the systolic blood pressure for which the difference was almost probable. There was, however, no significant difference for the diastolic blood pressure, although the figures in group III-IV were higher than in group 0-II, both for men and for women throughout. The filtration fraction, calculated for both sexes together, was practically of the same order of magnitude. As regards proteinuria and the urea nitrogen in the plasma, the figures were also consistently higher in group III-IV, as was the haematocrit figure. No statistically significant differences in these tests were, however, found between the two groups. It was not possible to make calculations for the creatinine and urea clearances since too few determinations were made in group III-IV. The mean figure and — if more than ten determinations were made in any of the groups — the standard error are nevertheless found in the table (in brackets) in order to give an indication of the direction in which these tests point as compared with the inulin clearance.

### The Systolic Blood Pressure

The figure of 210 mm Hg., used by the present writer as the borderline between the two groups compared, may appear to be a little too high. Several reasons nevertheless warrant this procedure since the material was too small to divide into several sub-groups, which would obviously have been more satisfactory. If the borderline is chosen at 210 mm Hg., the extreme elevations in blood pressure are included in the group above the limit and there is no risk of including cases of moderately elevated blood pressure. Another advantage is that an equal distribution is obtained in the two groups. Had a higher figure been chosen, the number of cases above this level would have been considerably smaller than is now the case.

Table XXXV. Mean (M) and standard error of the mean<sup>1</sup> ( $\pm$ ) of cases with essential hypertension, distributed according to systolic blood pressure above and below 210 mm Hg.

Determination	Systolic blood pressure			
	$\geq 210$ mm Hg		< 210 mm. Hg	
	Num-ber	M $\pm$ $\varepsilon$ (M)	Num-ber	M $\pm$ $\varepsilon$ (M)
Men				
Inulin clearance .....	10	79.5 $\pm$ (10.5)	10	102.7
Diodrast clearance .....	10	244.3 $\pm$ (40.6)	10	320.3
Filtration fraction .....	10	0.342 $\pm$ (0.025)	10	0.343
Creatinine clearance .....	3	102.0	2	108.0
Urea clearance .....	4	37.8	6	66.8
Effect. renal blood flow ....	10	447.2 $\pm$ (74.9)	10	599.4
Hematocrit .....	10	42.8 $\pm$ (2.7)	10	45.6
Proteinuria .....	9	0.100	10	0.130 $\pm$ (0.101)
Blood pressure { systolic ....	10	227.5 $\pm$ (4.0)	10	170.0 $\pm$ (6.5)
	10	138.0 $\pm$ (5.9)	10	101.0 $\pm$ (3.9)
Urea nitrogen, mg % .....	6	28.1	9	22.5
Women				
Inulin clearance .....	26	82.7 $\pm$ 3.6	16	104.9 $\pm$ 4.5
Diodrast clearance .....	26	252.0 $\pm$ 14.5	16	323.4 $\pm$ 17.2
Filtration fraction .....	26	0.340	16	0.336 $\pm$ 0.017
Creatinine clearance .....	8	84.0	5	124.6
Urea clearance .....	20	54.1 $\pm$ 4.8	12	74.3 $\pm$ (4.8)
Effect. renal blood flow ....	26	451.0 $\pm$ 28.1	16	566.7 $\pm$ 29.9
Hematocrit .....	26	43.58	16	42.88 $\pm$ 0.88
Proteinuria .....	26	0.169 $\pm$ 0.100	16	0.025 $\pm$ 0.011
Blood pressure { systolic ....	26	242.5 $\pm$ 4.8	16	179.7 $\pm$ 4.0
	26	132.7 $\pm$ 2.9	16	104.4 $\pm$ 2.5
Urea nitrogen, mg % .....	22	20.7	13	19.3 $\pm$ (2.4)
Both sexes				
Inulin clearance .....	36	81.8 $\pm$ 3.8	26	104.0 $\pm$ 3.7
Diodrast clearance .....	36	249.9 $\pm$ 15.0	26	322.2 $\pm$ 15.6
Filtration fraction .....	36	0.341 $\pm$ 0.011	26	0.339
Creatinine clearance .....	11	88.9 $\pm$ (7.2)	7	119.9
Urea clearance .....	24	51.4 $\pm$ 4.5	18	71.8 $\pm$ 3.9
Effect. renal blood flow ....	36	449.9 $\pm$ 28.4	26	579.3 $\pm$ 29.5
Hematocrit .....	36	43.36 $\pm$ 0.90	26	43.92
Proteinuria .....	35	0.151 $\pm$ 0.075	26	0.065
Blood pressure { systolic ....	36	238.3 $\pm$ 3.8	26	176.0 $\pm$ 3.6
	36	134.2 $\pm$ 2.6	26	103.1 $\pm$ 2.1
Urea nitrogen, mg % .....	28	22.3 $\pm$ 1.6	22	20.6

<sup>1</sup> v. foot note p. 129

Table XXXXV is a collocation of the results in the two groups. As in the earlier tables, the standard error of the mean was only calculated if the number of cases in both groups was over ten and — or if — it was evident that a difference could be anticipated. In other cases only the mean figure is given, but not the standard error. In respect of the creatinine clearance determinations there were not sufficient cases, either for each sex separately or for both sexes together, for a calculation. For both sexes together statistically significant differences could be shown for the inulin, urea and diodrast clearances and the effective renal blood flow with lower values in the group with blood pressure  $\geq 210$  mm Hg and a higher value for the diastolic blood pressure. The small difference found in the other tests was very slight or non-existent in some of them.

### The Diastolic Blood Pressure

With regard to the diastolic blood pressure the material was divided into two groups with 120 mm Hg as the borderline. The same points of view raised in connexion with the systolic blood pressure are applicable here. It is more difficult to classify cases according to diastolic than according to systolic blood pressure. Opinions are in agreement as regards the determination of systolic blood pressure and no difficulties are encountered in making such readings, but opinions are divided regarding the reading of the diastolic pressure. Some workers are of the opinion — and they are certainly correct — that the diastolic blood pressure, that can only be determined by auscultation, should be given for the blood pressure figure at which the arterial sounds change in character and become weaker. Others believe that the level at which the arterial sounds suddenly disappear give the correct figure. These facts explain why the diastolic pressure is more difficult to judge, particularly since different investigators use different methods in their readings. Were the difference between the figures constant when the arterial sound changes in character and becomes weaker or when it entirely disappears, this fac-

Table XXXXVI. Mean (M) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) of cases with essential hypertension, distributed according to diastolic blood pressure above and below 120 mm Hg.

Determination	Diastolic blood pressure			
	$\geq 120$ mm Hg		< 120 mm Hg	
	Number	M $\pm$ $\epsilon$ (M)	Number	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance .....	10	90.0 $\pm$ (8.4)	10	91.3
Diodrast clearance .....	10	271.8 $\pm$ (34.1)	10	292.8
Filtration fraction .....	10	0.353 $\pm$ (0.025)	10	0.332
Creatinine clearance .....	3	102.0	2	108.0
Urea clearance .....	4	56.8	6	54.2
Effect. renal blood flow ....	10	510.7 $\pm$ (63.8)	10	535.9
Hematocrit .....	10	46.2 $\pm$ (1.4)	10	42.2 $\pm$ (3.0)
Proteinuria .....	9	0.100	10	0.130 $\pm$ (0.101)
Blood pressure { systolic ....	10	222.5 $\pm$ (8.4)	10	175.0 $\pm$ (7.4)
	10	139.0 $\pm$ (5.4)	10	100.0 $\pm$ (3.4)
Urea nitrogen, mg % .....	6	22.2	9	26.4
Women				
Inulin clearance .....	25	86.0 $\pm$ 4.3	17	98.8 $\pm$ 4.6
Diodrast clearance .....	25	264.7 $\pm$ 16.8	17	300.5
Filtration fraction .....	25	0.338 $\pm$ 0.012	17	0.341
Creatinine clearance .....	9	89.8	4	121.8
Urea clearance .....	18	56.5 $\pm$ 5.2	14	68.4
Effect. renal blood flow ....	25	468.6 $\pm$ 31.0	17	534.1
Hematocrit .....	25	43.08 $\pm$ 0.77	17	43.65
Proteinuria .....	25	0.176 $\pm$ 0.104	17	0.024
Blood pressure { systolic ....	25	241.8 $\pm$ 5.4	17	184.4 $\pm$ 5.1
	25	134.6 $\pm$ 2.5	17	103.2 $\pm$ 2.0
Urea nitrogen, mg % .....	20	20.5 $\pm$ 1.1	15	19.8
Both sexes				
Inulin clearance .....	35	87.4 $\pm$ 3.8	27	96.0
Diodrast clearance .....	35	266.7 $\pm$ 15.2	27	297.7
Filtration fraction .....	35	0.342 $\pm$ 0.011	27	0.337
Creatinine clearance .....	12	92.8 $\pm$ (7.2)	6	117.2
Urea clearance .....	22	56.5 $\pm$ 4.6	20	64.1
Effect. renal blood flow ....	35	480.6 $\pm$ 28.3	27	534.7
Hematocrit .....	35	43.97 $\pm$ 0.70	27	43.11
Proteinuria .....	34	0.156 $\pm$ 0.077	27	0.063
Blood pressure { systolic ....	35	236.3 $\pm$ 4.5	27	180.9 $\pm$ 4.5
	35	135.9 $\pm$ 2.3	27	102.0 $\pm$ 1.8
Urea nitrogen, mg % .....	26	20.90 $\pm$ 0.91	24	22.29

tor would be of little importance. This is probably not the case. The higher and more stabilized is the pressure, the greater the amplitude apparently present between these two points. Since it is easier to determine the level at which these sounds disappear and it was, moreover, earlier the custom at the University Hospital in Upsala to read the diastolic pressure at this point, this rule has been followed in the present investigation.

On examination of Table XXXXVI it is seen that in grouping the material into two groups with a diastolic blood pressure of 120 mm Hg as the borderline, there were no statistically significant differences between the groups (except, obviously, as regards the blood pressure). It is seen from the table for both sexes together that there was a general tendency for all the figures to imply a greater impairment of renal function — except in the case of urea nitrogen, in which the conditions were reversed — in cases with a pressure  $\geq 120$  mm Hg. In the tables for men and women separately, this fact is not found throughout for all the tests. It can therefore be said that the only definite information obtained from this classification is that there was a significant difference in the case of the blood pressure, which is natural since this formed the basis of the classification. The reason for this dissimilarity as regards the results after grouping according to the height of the systolic and diastolic blood pressure is possibly to be sought in the method used for the reading of the diastolic pressure (v. the foregoing).

### Non Protein Nitrogen or Urea Nitrogen

As in the cases of chronic glomerulonephritis, the hypertensive patients were classified into those with normal non protein nitrogen or urea nitrogen and those with raised values. The borderline figure was the same as that given earlier (v. p. 17). The same principle was also applied, i.e. if there was any doubt concerning the group to which a certain patient belonged, the non protein nitrogen figure was the decisive factor. The material was found to fall into two

Table XXXXVII. Mean (M) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) of cases with essential hypertension, distributed according to non protein nitrogen and urea nitrogen.

Determination	Non protein nitrogen > 40 mg %		Non protein nitrogen $\leq$ 40 mg %	
	Urea nitr. > 20 mg %		Urea nitr. $\leq$ 20 mg %	
	Number	M $\pm$ $\epsilon$ (M)	Number	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance .....	10	72.9 $\pm$ (8.9)	10	109.3 $\pm$ (5.6)
Diodrast clearance .....	10	193.4 $\pm$ (26.2)	10	371.2 $\pm$ (24.3)
Filtration fraction .....	10	0.383 $\pm$ (0.028)	10	0.302 $\pm$ (0.023)
Creatinine clearance .....	1	72.0	4	112.5
Urea clearance .....	5	36.6	5	73.8
Effect. renal blood flow ....	10	348.9 $\pm$ (50.2)	10	697.7 $\pm$ (44.3)
Hematocrit .....	10	41.7 $\pm$ (2.6)	10	46.7
Proteinuria .....	9	0.200	10	0.040 $\pm$ (0.082)
Blood pressure { systolic ....	10	211.5 $\pm$ (13.0)	10	186.0
	10	126.0 $\pm$ (8.3)	10	113.0
Urea nitrogen, mg % .....	8	30.3	7	18.4
Women				
Inulin clearance .....	18	83.4 $\pm$ 5.8	23	98.0
Diodrast clearance .....	18	248.6 $\pm$ 18.7	23	306.3 $\pm$ 14.9
Filtration fraction .....	18	0.347 $\pm$ 0.016	23	0.332
Creatinine clearance .....	4	102.8	9	98.2
Urea clearance .....	15	58.0 $\pm$ 5.9	17	64.9
Effect. renal blood flow ....	18	435.0 $\pm$ 35.2	23	547.2 $\pm$ 25.9
Hematocrit .....	18	42.28 $\pm$ 0.84	23	44.04
Proteinuria .....	18	0.094 $\pm$ 0.053	23	0.135
Blood pressure { systolic ....	18	226.9 $\pm$ 10.1	23	213.3
	18	123.1 $\pm$ 5.1	23	122.0
Urea nitrogen, mg % .....	15	24.55 $\pm$ 0.94	20	16.95 $\pm$ 0.51
Both sexes				
Inulin clearance .....	28	79.6 $\pm$ 4.9	33	101.5 $\pm$ 3.0
Diodrast clearance .....	28	228.9 $\pm$ 15.6	33	326.0 $\pm$ 13.4
Filtration fraction .....	28	0.360 $\pm$ 0.015	33	0.323
Creatinine clearance .....	5	96.6	13	102.6 $\pm$ (5.5)
Urea clearance .....	20	52.7 $\pm$ 5.3	22	67.0 $\pm$ 3.9
Effect. renal blood flow ....	28	404.3 $\pm$ 29.4	33	592.8 $\pm$ 24.4
Hematocrit .....	28	42.1 $\pm$ 1.1	33	44.8
Proteinuria .....	27	0.130 $\pm$ 0.050	33	0.106
Blood pressure { systolic ....	28	221.4 $\pm$ 7.7	33	205.0
	28	124.1 $\pm$ 4.3	33	119.2
Urea nitrogen, mg % .....	23	26.55 $\pm$ 1.65	27	17.32 $\pm$ 0.53

<sup>1</sup> v. foot note p. 129

groups approximately equal in size. It was possible to make calculations for the majority of the tests both for men and women separately and for both sexes together (Table XXXXVII). It is seen that in the latter case the differences were significant between the two groups for the inulin and diodrast clearances and the effective renal blood flow. For men and women separately the figures for these tests also were throughout lower in the group with higher non protein nitrogen. In a number of tests, but not throughout, the differences were significant. The filtration fraction, for example, showed no significant difference but was consistently higher for each sex separately and for both sexes together when the non protein nitrogen was raised. This was also the case for the systolic and diastolic blood pressure.

### The Duration

In an attempt to ascertain whether the duration of essential hypertension has any relation to the disturbances in renal function demonstrated, a further grouping was made of the material on this basis. It would naturally have been best to make several sub-groups according to the age-distribution, but this was not possible. Hypertension of long or short duration was therefore the basis of the classification. The borderline was fixed at three years, which appears to be reasonable since the condition is chronic and often lasts for many years. The results of this grouping are seen in Table XXXXVIII. It is seen that there was only one statistically significant difference for the sexes together, i.e. for the systolic blood pressure which was higher in the group of patients in which the duration of the disease was over three years. The same difference was statistically probable in the females. No differences could be calculated in the males since the material was too small. The tendency was, nevertheless, the same. There was no significant difference as regards the diastolic blood pressure, although the figures throughout showed the same tendency as for the systolic blood pressure.

Table XXXXVIII. Mean (M) and standard error of the mean<sup>1</sup> ( $\epsilon$ ) of cases with essential hypertension, distributed according to duration of the disease.

Determination	Duration of the disease			
	> 3 years		< 3 years	
	Number	M $\pm$ $\epsilon$ (M)	Number	M $\pm$ $\epsilon$ (M)
Men				
Inulin clearance .....	11	98.5 $\pm$ (7.2)	9	82.0
Diodrast clearance .....	11	304.9 $\pm$ (33.5)	9	254.7
Filtration fraction .....	11	0.348 $\pm$ (0.028)	9	0.336
Creatinine clearance .....	2	97.5	3	109.0
Urea clearance .....	6	59.0	4	49.5
Effect. renal blood flow ....	11	561.5 $\pm$ (61.5)	9	476.7
Hæmatocrit .....	11	45.18 $\pm$ (1.74)	9	43.00
Proteinuria .....	10	0.060 $\pm$ (0.034)	9	0.178
Blood pressure { systolic ....	11	205.9 $\pm$ (9.2)	9	190.0
	11	121.8 $\pm$ (6.5)	9	116.7
Urea nitrogen, mg % ....	9	21.2	6	30.1
Women				
Inulin clearance .....	16	82.4 $\pm$ 5.5	25	96.8 $\pm$ 3.9
Diodrast clearance .....	16	245.3 $\pm$ 19.6	25	301.5 $\pm$ 15.0
Filtration fraction .....	16	0.346 $\pm$ 0.020	25	0.334
Creatinine clearance .....	4	99.3	8	100.4
Urea clearance .....	11	54.1 $\pm$ (5.9)	20	65.8
Effect. renal blood flow ....	16	436.1 $\pm$ 34.7	25	533.2 $\pm$ 28.3
Hæmatocrit .....	16	43.44 $\pm$ 0.94	25	43.16
Proteinuria .....	16	0.113 $\pm$ 0.058	25	0.120
Blood pressure { systolic ....	16	238.8 $\pm$ 8.7	25	205.6 $\pm$ 7.0
	16	128.8 $\pm$ 4.0	25	118.4 $\pm$ 3.9
Urea nitrogen, mg % ....	12	21.3 $\pm$ (1.6)	22	19.8
Both sexes				
Inulin clearance .....	27	89.0 $\pm$ 4.6	34	92.9
Diodrast clearance .....	27	269.6 $\pm$ 18.4	34	289.1
Filtration fraction .....	27	0.347 $\pm$ 0.016	34	0.335
Creatinine clearance .....	6	87.0	11	102.7 $\pm$ (9.6)
Urea clearance .....	17	55.8 $\pm$ 4.9	24	63.1
Effect. renal blood flow ....	27	487.2 $\pm$ 33.9	34	513.3
Hæmatocrit .....	27	44.15 $\pm$ 0.90	34	43.12
Proteinuria .....	26	0.092	34	0.135 $\pm$ 0.078
Blood pressure { systolic ....	27	225.4 $\pm$ 7.0	34	201.5 $\pm$ 6.1
	27	125.9 $\pm$ 3.5	34	117.9
Urea nitrogen, mg % ....	21	21.2 $\pm$ 1.2	28	22.0

<sup>1</sup> v. foot note p. 129



### Summary and Discussion

With the tests used here, it was possible to demonstrate a disturbance of renal function in 62 patients with more or less advanced essential hypertension in the following respects. The clearances were lower for inulin, diodrast, creatinine and urea, and the figures for the effective renal blood flow and the haematocrit were also lower. As a result of the proportionately greater decrease in the diodrast than in the inulin clearance, the filtration fraction was higher. All the differences obtained in comparison with the normal material were statistically significant with the exception of the haematocrit figure for which the difference was probable, and the level of urea nitrogen for which no difference could be demonstrated at all.

The frequency of the various clinical symptoms is given in Table XXXXII (v. p. 178).

When a calculation was made of the correlation between two different tests, such a correlation appeared to exist between the inulin and creatinine clearances, the inulin and diodrast clearances and also possibly between other tests as well (v. Table XXXXI, p. 177).

After grouping the material according to various clinical symptoms, the following additional remarks may be made.

In a classification according to the *retinal changes*, the group of patients with severe changes (corresponding to Keith's groups III-IV) showed more impairment of renal function as judged by the inulin and diodrast clearances. They also had a higher systolic blood pressure. These differences are statistically significant. The changes in the creatinine and urea clearances pointed in the same direction, as did the increase in diastolic blood pressure and urea nitrogen, although the differences were not even probable.

If the material was classified according to the level of the *systolic blood pressure*, more impaired function was found in the group with high pressure, with significantly lower values for the inulin, diodrast and urea clearances and the effective renal blood flow.

If the patients were classified according to the level of the *diastolic blood pressure*, there were no statistically significant differences between the two groups, but, nevertheless, a tendency throughout to more impaired function with higher pressure.

The cases with raised *non protein nitrogen*, considered as a whole, naturally showed more pathological functional tests than the group with a normal non protein nitrogen level. This is clearly due to the fact that the non protein nitrogen only begins to rise when there is a certain degree of renal lesion (v. Chapter VIII).

In all the groupings of the material, the filtration fraction was somewhat higher in that group with the most severe clinical symptoms (more severe retinal changes, higher blood pressure, longer duration, etc.). The differences were not however statistically significant on the basis of any of the classifications. Nevertheless the filtration fraction was higher in the group with increased non protein nitrogen than according to any other classification of the material. This circumstance will be discussed further in the following.

Finally, if the *duration* of the hypertensive disease is taken into consideration, the statistical calculation showed that there was no statistically significant difference in renal function between the two groups above and below 3 years (see p. 188).

It is thus evident that I have found a — in certain respects strong — correlation between the usual clinical symptoms and the changes in renal function. It is moreover seen from Table XXXVIII that the damage of renal function in those groups in which it is severe is also usually linked with an increased mortality in the same groups, although not more than approximately 20 per cent of the deaths were caused by uraemia. The mortality was thus highest in the patients with severe retinal changes, followed by those who showed an increase in non protein nitrogen. The duration of the disease, which was shown to have no significant importance as regards the results of the tests, occupies however the third place as regards the mortality instead of occupying the last one,

which could be expected according to the results of the tests. The most natural explanation is that the longer the duration of the disease, the greater the risk of a fatal outcome. It would obviously be possible to apply this explanation to the other classifications of the material. The problem was nevertheless a different one here, i.e. what information is given by the *actual* clinical symptoms, the duration excepted, concerning the functional state of the kidneys? It must be borne in mind that azotaemia was not the cause of death in all the cases but only in just over 20 per cent. Cardiac or cerebral crises were responsible for 80 per cent of the mortality. The only definite fact proved by the mortality figures in this group is that in this small material malignant hypertension did not occur on any large scale during the first years of the duration of the hypertensive disease. This same fact, seen from another point of view, also throws some light on the results of the renal functional tests. Moreover, the parallelism between the mortality and the results of the functional tests is very marked in the present material. Of the clinical symptoms manifested by the patients on examination, it has been found that retinal changes occupy a unique position. Thus not only do they indicate a poorer prognosis, as was earlier known (v. e.g. Wagener and Keith 1939) but — as earlier pointed out by Hilden (1946) among others — they also indicate a greater impairment of renal function despite the fact that the majority of deaths within this disease are due to other causes than uraemia. It is thus natural to assume that the same kind of changes in the blood vessels that give rise to changes in the fundi are also manifest in the kidneys. If, however, changes are found in both these organs, there is reason to believe that the vascular disturbances are not localized to these sites alone but are more diffusely spread throughout the entire organism and the high mortality within this group is therefore entirely reasonable. Further support for this interpretation is given by the investigations of Kernohan, Anderson and Keith (1929). On microscopical examination of the striated muscles they found that in benign hypertension there were no or only insignificant

changes in the blood vessels, whereas in malignant hypertension — 23 cases, all with retinal lesions were examined — there was a thickening of the vessel walls throughout as well as a stricture in the walls of the muscular arterioles.

Further analysis of two facts seems desirable, i.e. the lack of correlation between the increased diastolic blood pressure on the one hand and the results of the tests on the other and also between the former symptom and the filtration fraction.

The increased level of the diastolic blood pressure is generally considered to be a reliable basis for the diagnosis of essential hypertension as it indicates increased peripheral resistance. There is no doubt that this is the case for the *diagnosis* of essential hypertension, but another question is obviously whether the actual value gives any information regarding the degree of the disturbance in renal function or the prognosis of the disease. Opinions on this matter have varied. Friedman, Selzer and Rosenblum (1941) showed in their material that there was a very good correlation between the filtration fraction and the diastolic blood pressure: the higher the diastolic pressure the higher the filtration fraction and the smaller the effective renal blood flow. If, however, a closer study is made of their paper, it is seen that their material (32 individuals, of whom 8 were women) was divided into four groups according to the level of the diastolic pressure, i.e. 60-80, 80-100, 100-120, and 120-140 mm Hg. The first group with a diastolic pressure between 60 and 80 mm Hg can scarcely be considered to suffer from essential hypertension, nor the group with a pressure between 120 and 140 mm Hg, in which all six patients suffered from coarctation of the aorta, a disease which has certainly nothing to do with *essential* hypertension. If these two groups are eliminated the forementioned authors' data are not informative as only 23 cases are left. Hilden (1946) also investigated in his material whether there was any correlation between the diastolic pressure and the filtration fraction and came to the conclusion that none could be demonstrated. The present writer reached the same conclusion (v. fig 12). In view of the fact that two different workers,

using different methods and having a total material of more than 120 patients, obtained practically the same results, it appears very probable that the question of the relation of the diastolic blood pressure to the filtration fraction — and probably also to the actual state of renal function — can be considered as fairly well settled. No established correlation

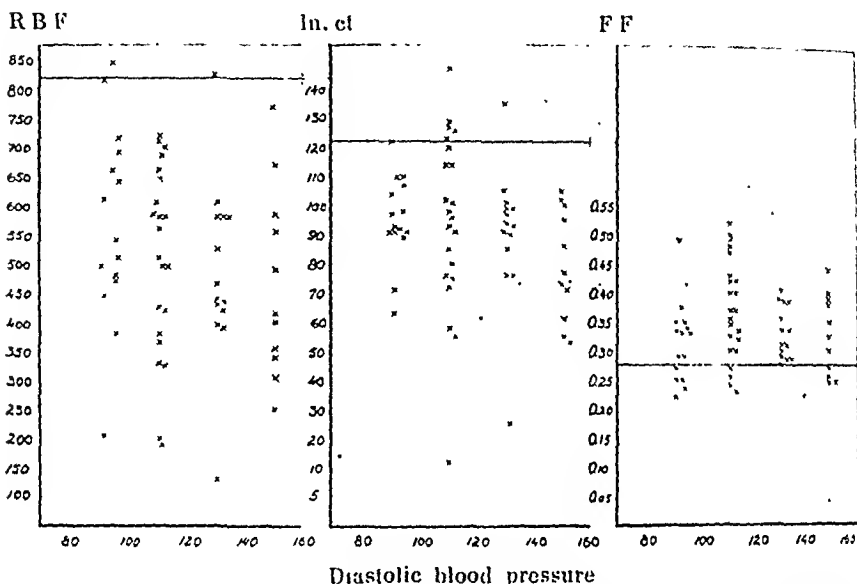


Fig 12. Diagram showing the effective renal blood flow, inulin clearances and filtration fractions in patients with essential hypertension in relation to the level of the diastolic blood pressure. The thin horizontal lines mark the normal values.

thus seems to exist. On the other hand, a correlation was found between the state of renal function and the systolic blood pressure. This could possibly be due to the fact that all cases of severe arteriosclerosis were included in the group with a high systolic pressure. If in addition to the functional or organic constriction of the arterioles present in essential hypertension there is also a more pronounced arteriosclerosis of the blood vessels, particularly also the aorta, the elasticity of the aorta then obviously being decreased, this must result in a considerable rise of the systolic pressure whereas the diastolic is unaffected. Another reason for this lack of correlation between renal function and the diastolic blood pres-

sure can perhaps be sought in the fact mentioned earlier, i.e. the method of reading this pressure.

Finally, as regards the filtration fraction, this was raised in the large majority of cases but not in every one as e.g. Goldring and Chasis (1944) have stated. The figures for this fraction, grouped according to decreasing inulin clear-

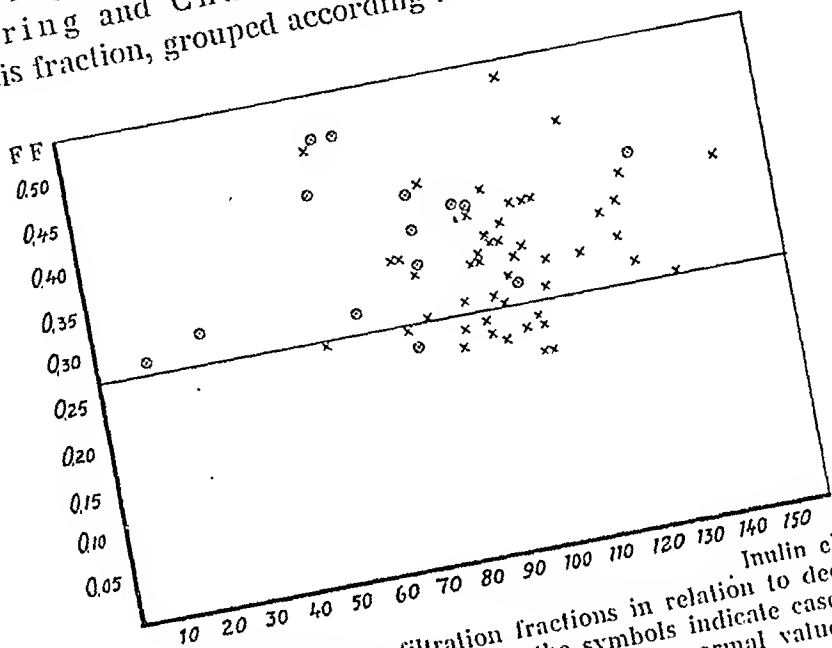


Fig 13. Diagram showing the filtration fractions in relation to decreasing inulin clearance values. The rings round the symbols indicate cases which are deceased. The horizontal line indicates the normal value for the filtration fraction.

ance values, are given in fig 13. The distribution of the patients with high and low filtration fractions can also be seen in this graphical survey. It is seen that the lower the inulin clearance the higher the mortality rate although, as has been pointed out several times earlier, only approximately 20 per cent of the patients died of uraemia. It is perhaps also possible to trace some tendency to a higher filtration fraction with lower inulin clearance but the same prognostic possibilities are not present here as in the case of chronic glomerulonephritis (v. Discussion, Chapter V).

Another complicating factor in the present material is the age. It was pointed out earlier that age has some significance for renal function. This is shown, amongst other signs, by

a higher concentration of urea nitrogen with rising age (MacKay and MacKay 1927, Lewis and Alving 1938) in a decreased urea clearance (Lewis and Alving 1938) and a decreased diodrast clearance (Hilden 1946). The last-mentioned writer also showed that the diodrast clearance decreases proportionately more than that of urea and the

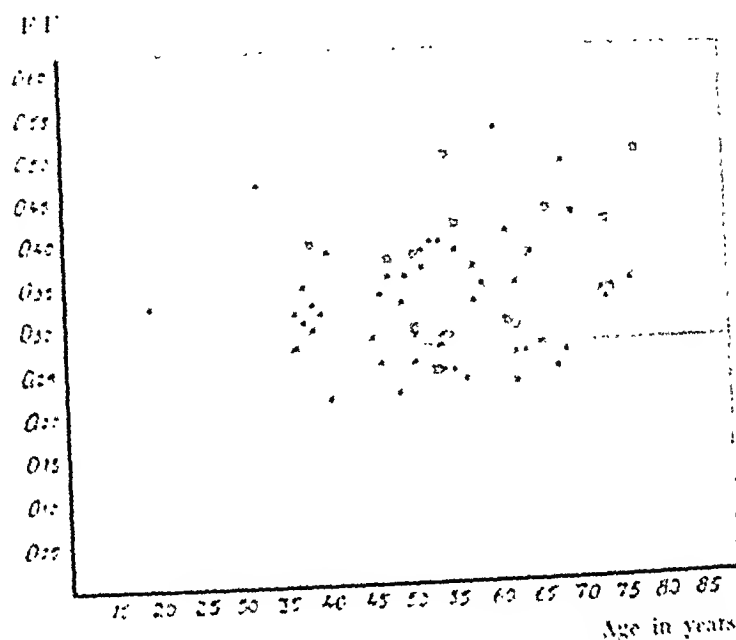


Fig 14 Diagram showing the filtration fractions in relation to the age of the patient. As to the symbols etc. v. fig 13

filtration fraction thus probably increases. Under such conditions, the same disturbance in renal function occurs in rising age - and particularly in the age groups 60-80 years - as described in essential hypertension. Hilden also pointed out this fact.

To return to the present material, it is evident that the majority of cases with very high filtration fractions are to be sought among the patients over the age of 60 years. It is also in this group that we find a proportionately lower inulin clearance and, naturally, a higher mortality rate. In order to elucidate certain of these points, all the filtration fractions in relation to rising ages of the patients are given

graphically in fig 13. It is nevertheless seen from this graph that even the majority of patients below the age of 60 years also showed figures for the filtration fraction above the normal level.

In the various groupings of the material according to the degree of the clinical symptoms, no large difference was as a rule found in the filtration fraction in the sub-groups, with the exception of the classification according to the concentration of non protein nitrogen. In the group with non protein nitrogen  $> 40$  mg per cent, this fraction was considerably higher than in the group with non protein nitrogen  $\leq 40$  mg per cent, although the prognosis for the former group, according to the mortality rate and also the results of the functional tests, was better than in the group with retinal changes. It is impossible to say whether this is only due to random variation or whether it could indicate that with an increase of non protein nitrogen a certain compensation mechanism is set up in the kidney, manifested, for example, in an increased tonus in the efferent arterioles. The filtration pressure would then rise and a larger filtrate occur. This is as yet only a theoretical possibility. The whole question of increased tonus in the efferent arterioles will be discussed in the Chapter: General Discussion.



## CHAPTER VII.

### ONE FUNCTIONING KIDNEY

The kidneys, in common with several other organs, have a reserve power which makes possible a considerably greater effectivity than normally called upon. This is demonstrated by the fact that it is possible to perform unilateral extirpation of the kidney without any fatal risk in otherwise healthy, and not too old individuals. The functional capacity of a single kidney is thus sufficient for the needs of the body. In this connexion two questions can be asked.

1. How large is the functional capacity of the remaining kidney immediately after unilateral nephrectomy?

2. How large is the functional capacity of the remaining kidney later on, i.e. does hypertrophy or hyperplasia occur?

#### Earlier Investigations

Attempts have been made to solve the latter problem experimentally on animals. Lorenz (1885) thus found that the increase in volume occurring in one kidney when the other is extirpated depends chiefly on an increase in the volume of the cortex as a result of hypertrophy. In young animals a slight hyperplasia of the glomeruli and the convoluted tubules also occurs. The insignificant increase in the volume of the medulla would depend solely on dilatation of its tubules and no increase in the number or size of its cells occurs. Galeotti and Villa-Santa (1902) obtained the same results, but they appear to attach more importance to the hyperplasia of the glomeruli in young animals than did Lorenz. Oliver (1924) also found that

the hypertrophy occurring in a remaining kidney was most marked in the convoluted tubules, 32 per cent being localized to them, 10 per cent to the glomeruli, 9 per cent to the ascending limb of Henle's loop and only 3 per cent to the collecting tubules. Wrele (1943) showed on a large material that an increase in weight also occurs in the remaining kidney of one-year-old white mice, castrated a few weeks after birth and later nephrectomized. He did not, however, discuss whether this increase was due to hypertrophy or to hyperplasia or to both factors. This increase in the remaining kidney was approximately 40 per cent one to several months after unilateral nephrectomy. Wrele considered that he was able to exclude the possibility of this being caused by hyperaemia or an increased content of water. Moore and Lukianoff (1929) examined the open glomeruli in normal rabbits following the injection of Janus green B into the renal artery. 44-48 per cent of the glomeruli of the normal animals contained circulating blood at any one moment whereas this number rose to 91-99 per cent in rabbits on whom unilateral nephrectomy had been performed. The writers concluded that, at any rate during the first ten days after nephrectomy, a certain compensation occurs by an increase in the number of open glomeruli in the remaining kidney. Moore (1929), on the contrary, demonstrated in the white rat that unilateral nephrectomy during the period of active nephrogenesis that occurs in this animal even a few days after birth had no effect on the total number of glomeruli formed in the remaining kidney.

Moberg (1936) made a thorough investigation in his monograph of these conditions in the white rat. He came to the conclusion that following nephrectomy or a still greater reduction of the renal parenchyma an anatomical hypertrophy occurs in the remaining part, but that this hypertrophy does not always result in functional improvement. He was of the opinion that in considerable loss of the renal parenchyma, an anatomical hypertrophy implies a progressing injury to the remainder of the kidney. The question is therefore not *if* but *how long* a certain residue of the

kidney can retain sufficient capacity to maintain life. According to Moberg's investigations, this anatomical hypertrophy is conditioned by 1) an increasing enlargement of the glomeruli but otherwise no change in their structure 2) considerable widening of the convoluted tubules with an early onset of degenerative lesions.

It has long been known that an increase in the weight and volume of one kidney also occurs in man if the other is lacking or is extirpated. In, for example, *Handbuch der speziellen Pathologischen Anatomie* (Henke and Lubarsch 1925) these questions are treated by Gruber and Lubarsch. After a critical survey of the literature, these writers came to the conclusion that the increase in volume in the larger kidney in renal hypoplasia or in the only kidney in aplasia and after nephrectomy is dependent on the following two factors. 1) Hypertrophy, which can result in as much as 150 per cent of the normal size and which occurs both in congenital and acquired conditions. 2) Hyperplasia (and hypertrophy) when the condition arises during foetal life.

In addition to these anatomical investigations, various functional investigations following nephrectomy have been made. Shilling (1905), working with the rabbit, found no change in the volume of urine but in a few animals shortly after operation, a lower concentration of NaCl in the urine was noticed after large oral doses of NaCl and restriction of fluid. Ferron (1910) found under normal conditions no change in the excretion of chloride and urea in the rabbit following nephrectomy, but no tolerance tests were performed. Ambard and Papin (1909) found the same results as regards the urea concentration capacity. After urea tolerance tests, however, Addis and Watanabe (1916) found a lower quotient in the Addis ratio in nephrectomized than in normal animals. Addis, Myers and Oliver (1924) came to the same results and thus considered that they had confirmed their hypothesis that the ratio 
$$\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 g of plasma}}$$
 varies in direct proportion to the weight of the renal tissue. Holb-

weg (1915) found a slight increase of non protein nitrogen in the blood one or two days after nephrectomy, as did Karsner, Bunker and Grabfield (1915) in one dog, but not in another. This change, they believe, was nevertheless probably caused by the surgical intervention *per se* and was not connected with any changes brought about by nephrectomy.

Rhoads, Alving, Hiller and Van Slyke (1934) explanted one kidney in a dog and left the other *in situ*. This operation resulted in no change in the urea clearance. Subsequent extirpation of the non-explanted kidney gave a mean decrease of urea clearance of 36 per cent. The fact that the clearance fell to 64 per cent and not to 50 per cent showed that an increased function had occurred in the remaining kidney. This was evident during the first weeks following nephrectomy. During further observation of the remaining explanted kidney for two years, the clearance showed fairly constant or somewhat rising figures. In another investigation by the same group of workers (Van Slyke, Rhoads, Hiller and Alving 1934) it was calculated that after extirpation of one kidney in the dog, the renal blood flow increased by 68 per cent, the consumption of oxygen by 81 per cent and the urea clearance by 43 per cent, calculated on the pre-operative figures for one kidney. The increase reached its maximum within one month after the operation. A similar investigation was made somewhat later by Levy and Blalock (1938) on 12 dogs. In this investigation unilateral nephrectomy was accompanied by a slowly progressing increase in the renal blood flow through the kidney and a parallel increase in the oxygen consumption. This was somewhat more rapid during the first month than later and reached approximately the combined blood flow of the two kidneys at the end of the third month.

The question then arises whether a similar adaptation occurs *in man*. In order to answer it, we must rely on investigations made on individuals who have one functioning kidney or who have been nephrectomized. Few such investigations have, however, been made.

Ellis and Weiss (1933) investigated 12 patients between the ages of 16 and 52 years, 8 days to 12 years after unilateral nephrectomy. In nine of them the remaining kidney was in all probability normal. Determinations of the urea and creatinine clearances were made, as well as the concentration and dilution test according to Volhard. Two patients were examined immediately after the operation and the clearance values were then found to be decreased. A definite decrease was also found in five of the remaining patients and normal values in two. The writers came to the following conclusions. A decrease in renal function can, but does not always, take place following nephrectomy. The three patients who suffered from complications affecting the other kidney (pyonephrosis in two cases and hypertension in one) all showed decreased figures.

Friedman, Selzer, Kreutzmann and Sampson (1942) determined the inulin and diodrast clearances in five patients both before and 13-19 and 56-135 days after nephrectomy. All these patients, however, suffered from hypertension. The results of their investigation are found in Table XXXXVIII.

They sum up their conclusions of this investigation by stating, *inter alia*, that an increase in the renal blood flow and in glomerular filtration occurred in the remaining kidney in all the cases. This statement does not, however, agree with the figures in the table, at any rate for Cases 2 and 5, and data regarding the results of the investigation 13-19 days after the operation are lacking for Case 1. It can be seen from the results of the functional tests 56-135 days post-operatively that in two of the patients there was an increase in the renal blood flow compared with the figure before the intervention, practically the same figures as previously in two patients and a considerably lower figure in one case. An increase in the inulin clearance occurred in two cases, whereas three patients showed lower figures than prior to nephrectomy. It is seen from the table that all the patients were young and that the disease in the extirpated kidney was of long standing. Moreover, judging from the x-rays before the operation, the

Table XXXXVIII

The cases of Friedman et al (1942)

No. of Pat.	Diagnosis	Age	Before nephrectomy				13 — 19 days after op.				56 — 135 days after op.			
			Effective renal blood flow	Inulin clearance	B. P.		Effective renal blood flow	Inulin clearance	B. P.		Effective renal blood flow	Inulin clearance	B. P.	
1.F.P.	Atrophic left kidney	24	720	83	150/100		—	—	—		965	133	160/100	
2.C.S.	Extensive hydro-nephrosis left kidney	34	522	118	155/120		590	74.5	130/90		588	97.3	128/90	
3.J.G.	Atrophic left kidney	18	665	114	220/135		1040	90	150/90		875	75	160/110	
4.G.B.	Cicatricial tissue surrounding left kidney	34	1073	78	170/95		1040	63.5	130/85		1025	103.8	150/90	
5.M.F.	Calculus in left kidney with obstruction of urine flow	34	675	132	195/135		381	68.7	170/120		422	76	185/120	

healthy kidney was enlarged in all the cases and hypertrophy had evidently taken place over a long period.

Welsh, Wellen and Taylor (1944) published the results of clearance tests in two pregnant women 15 days to 40 months after nephrectomy. One of them had suffered from an adenocarcinoma that had destroyed the entire kidney and the other had a calculus in one of the kidneys. In the former case there was no increase in the tubular excretion ( $T_{mD}$ ) the filtration rate or the renal blood flow post-operatively; the figures were approximately 70 per cent of the normal ones. In the latter case the corresponding figures rose gradually after the operation from approximately 50 per cent to 70 per cent after a few years. The writers assume that in the first patient any hypertrophy possible had already taken place before the operation since the extirpated kidney had ceased to function a long time previously. In the second patient, on the other hand, hypertrophy of the remaining kidney had started after the operation, since both kidneys had functioned prior to it. In neither of the cases, however, did the clearance attain normal figures but remained at about 70 per cent; thus full compensation did not occur.

Köhler (1944) in a follow-up investigation of nephrectomized patients, gives the results of creatinine clearance tests in 56 cases. One of them had a clearance of 200 ml/min, 13 a clearance between 150 and 200 ml/min, 24 between 100 and 150 ml/min, 12 between 70 and 100 ml/min and 6 between 50 and 70 ml/min. It is seen that if the borderline for normal creatinine clearance is fixed at 100 ml/min, 18 of the 56 patients investigated, i.e. 32 per cent, had lowered creatinine clearance figures. His table shows that the younger the patients the higher were the clearance figures and the older they were the lower the figures. This is in agreement with the assumption that renal function is better compensated in young patients after nephrectomy whereas there is less possibility of an increase of function in the remaining kidney in older persons. Leander (1945) in a paper on the importance of renal functional tests in urology, gave the inulin and diodrast clearance figures for a 22-year-old patient suf-

fering from renal tuberculosis. Before nephrectomy the inulin clearance was 73 ml/min and the diodrast clearance 397 ml/min. Eighteen days after the operation the corresponding figures were 67 and 251 ml/min. The non protein nitrogen was 39 mg per cent before the operation and 24 mg per cent after. No data are given for the function in this case before the disease (which could hardly be expected). Data are given in the same paper regarding the inulin clearance in a 24-year-old patient who underwent resection of one-third of the left kidney in 1941. Nephrectomy of the right kidney was performed in 1942, both operations on the grounds of renal tuberculosis. After the latter operation — no mention is made of how long after — the inulin clearance was 65 ml/min. and two years later (1944) it was 73 ml/min. Josephson (1947) also published data on the clearance figures for inulin, creatinine, diodrast and diodrast  $T_m$  for a patient 14 days after nephrectomy. They were then approximately 50 per cent of the normal figures.

## Nephrectomy from the Theoretical Viewpoint

Nephrectomy is as a rule performed to extirpate a kidney that has for some time been the site of pathological lesions, for example, calculus, hydronephrosis, a tumour, etc. and the other kidney has thus successively been forced to assume some part of the function of the diseased one. It is only owing to injuries by accidents that nephrectomy of a previously healthy kidney is made and such interventions are obviously uncommon. They can actually be regarded as physiological experiments to determine the reserve capacity of the kidney. Since they are relatively uncommon, we have no extensive knowledge of the conditions following such an intervention. Nevertheless, it can be considered as established that in such cases the composition of the blood is not perceptibly changed and that the excretion of urine takes place to an approximately normal extent, i.e. normal volume and concentration.



The various theoretical possibilities of an adaptation when one kidney is suddenly forced to replace two in a satisfactory manner will be discussed before giving an account of the cases.

If one kidney is removed, the first consequence is that the content of waste products in the blood increases. As a result, the remaining kidney should mobilize its reserve power, and function to approximately double its normal extent. Theoretically this can take place in various ways.

We assume that the quantity of waste products delivered to the blood per time unit is denoted as  $\Delta$ , the quantity of fluid that during the same time is eliminated from the blood is denoted as  $v_f$  and the concentration of the waste products in this fluid as  $c_f$ . A certain reabsorption (and rediffusion) of water and other substances, that are filtered out through the glomeruli, then takes place in the tubules. If we assume that the quantity of fluid reabsorbed or rediffused is  $v_r$  and that its concentration of waste products is  $c_r$ , we must get  $\Delta = v_f \cdot c_f - v_r \cdot c_r$ . This can be said to be a general and schematic formula for renal function.

If it is assumed that the concentration in the blood of a certain substance is constant before and after nephrectomy, this implies either that reabsorption or rediffusion is less, or that the filtration increases, resulting in both instances in an unchanged volume of urine (obviously, both processes can take place at the same time). The difference between  $v_f \cdot c_f$  and  $v_r \cdot c_r$  must, in other words, be as large as before the operation.

1. With regard to an *increase in filtration*, this can be envisaged as brought about in several ways.

a) If normally 50 per cent of the nephrons did not function but all were active after nephrectomy, this would imply an approximate doubling of the filtration. This probably takes place in the frog, but we have certain reasons to assume that this mechanism does not occur in man (v. the following).

b) Filtration can also increase through an increased blood supply to the glomeruli. This would then take place by means of a widening of the afferent blood vessels. If the rate

of the blood flow is assumed to be unchanged, these blood vessels must then increase their diameter by  $\sqrt{2}$ . A moderate increase in diameter has thus a very considerable effect on the blood supply.

c) Filtration in the glomeruli can also be increased by a constriction of the efferent arterioles, which impedes the flow and thus raises the filtration pressure. Peripherally of this constriction, i.e. in the vessels supplying the tubules, this pressure would then be lower.

2. If we consider the other possibility, i.e. unchanged filtration but a *decrease in reabsorption or rediffusion*, the conditions are more complicated.

As regards a decrease in reabsorption, this can be thought to take place in various ways.

a) In the measure that reabsorption is an expression of an active process, it can obviously increase or decrease through an increase or a decrease in the necessary stimulation. We know, for example, that various hormones can influence this process and cause changes in reabsorption.

b) In addition to active reabsorption, there is with all probability also a more passive rediffusion of certain substances through the tubules. A decrease in the rediffusion could be caused by the fluid in the tubules being less concentrated. The lower the concentration of the tubular urine, the smaller the difference in concentration between it and the blood circulating around the tubules with a resulting decrease in rediffusion. Naturally, the speed at which the fluid passes through the tubules could also influence the process, and consequently an increase in the filtration rate would cause a decrease in the reabsorption from this point of view (v. Chapter General Discussion, p. 220).

3. In renal function we are not only dealing with these three functions, i.e. filtration, active reabsorption and rediffusion, since an active *secretion* also takes place through the tubules and this can naturally increase or decrease. A change in this function can be caused by:

a) An increased blood flow to the tubules thus making

increased activity possible. A decreased blood flow should *per se* have the opposite effect, but this is not necessarily the case since the capacity of the tubules is dependent on the amount of excretory substance that is offered to them per time unit. If, for example, the concentration in the blood of the substance excreted is increased at the same time that the flow of blood is decreased, excretion can perhaps take place as effectively as if the blood flow increased at a lower concentration.

b) Other factors of more or less unknown nature may afford an adequate stimulus to the tubular cells. Hormones have been discussed in this connexion. Although we know very little about such factors it is quite possible that they play a greater rôle than the aforementioned factors.

The results of the investigations published earlier will be discussed in the following against the background of these theoretical viewpoints.

As regards the question of whether the remaining kidney, after nephrectomy, could increase its function by making use of some *nephrons not previously functioning*, some writers consider that this mechanism comes into action. This opinion is based on direct observations of the glomerular activity in the frog and has later been extended by inference to higher animals and to man. It is nevertheless shown by the investigations of Smith and Shannon and others that if 50 per cent of the renal parenchyma is removed, the  $T_m$  for various substances (glucose, diodrast, etc.) is also decreased by 50 per cent. These writers are of the opinion that no inactive nephrons are present in man.

If this were the case, the decrease should be less, in similarity with the conditions in the frog in which the inactive nephrons start to function. The question obviously arises whether the forementioned determinations are sufficiently reliable to exclude the possible existence of inactive nephrons. It nevertheless appears very probable that this mechanism does not exist in man.

The other question that must be discussed is that of the *volume of the filtrate* following nephrectomy. There are few

statements in the literature on this subject and no data appear to exist concerning the filtration rate in the remaining kidney when an earlier healthy or practically healthy kidney has been extirpated. The few determinations made are in respect of the filtration rate in the remaining kidney when nephrectomy has taken place owing to a pathological condition of more or less long duration. It is obvious that under such conditions the remaining kidney was able successively to adapt itself to the decrease of function in the affected one. In the few such cases in which filtration determinations were made, the filtration rate was 50-90 per cent of the normal value.

The third question is that of *reabsorption*. According to many authors (v. Chapter I) reabsorption of waste products — or perhaps more adequately expressed, of urea — consists in normal individuals of approximately 40-70 per cent of the filtered amount. There are, however, no statements in the literature regarding the conditions following nephrectomy.

In view of the foregoing, it must be considered that an increase in the filtration rate and in the blood flow to the remaining kidney in fact is insufficient to explain how one kidney can perform the work of two.

If we consider an other alternative, i.e. that the single kidney more effectively frees the blood, this can appear somewhat strange at first sight. We will consider the process in the case of one particular waste product. Under normal conditions, a considerable proportion of the filtered urea — varying between 40 and 60 per cent — returns to the blood. This is mainly conditioned by the fact that the glomerular filtrate, which is normally approximately 120 ml/min, is concentrated to such an extent during its passage through the tubules that the final urine flow is only 1 ml/min. The difference in the concentration of the urea in the tubular urine and that in the blood outside the tubular cells is therefore considerable and it is understandable that part of the urea rediffuses, or is possibly even actively reabsorbed. A proportion of the urea leaves the tubular urine in one — or both — of these ways. Otherwise, owing to its osmotic pressure, it would

inhibit the reabsorption of water, and the flow of urine would be very high.

If one kidney has to work alone and its filtration is maintained at the same level as when both kidneys were functioning, the glomerular filtrate must be concentrated to a lesser extent, i.e. less water must be reabsorbed in order to keep the flow of urine within the same limits as prior to nephrectomy. The difference in the concentration of the tubular urine and in the surrounding blood is smaller and less urea leaves the tubular urine. The excretion of urea from the single kidney becomes greater than it was earlier from each kidney separately when both kidneys were functioning. How great a compensation can be afforded by this mechanism? If we assume that 50 per cent of the filtered urea is normally rediffused (or reabsorbed) a decrease in the filtration by one-half (= removal of one kidney) would imply that in order for the excretion of urea to be the same, the entire filtered quantity of urea is excreted in the urine. In other words, there is no rediffusion. Obviously, this cannot be the case, since as long as the flow of urine remains within normal limits — and this is so even if only one kidney is present — the glomerular filtrate must be very substantially concentrated and some part of the urea thus return to the blood. Other compensatory mechanisms must therefore come into force, of which two kinds can be envisaged: 1) an increase in the concentration of urea in the blood, 2) an increase in the size of the glomerular filtrate.

An increase in the concentration of urea in the blood from 15 mg per cent to 20 mg per cent would imply that 25 per cent of the filtered quantity of urea could be reabsorbed. An increase of 25 per cent in the rate of the glomerular filtration (from 60 to 75 ml/min for the one kidney) on the other hand, would mean that 20 per cent of the urea would return to the blood. If both these occurrences took place simultaneously, the reabsorption would be 40 per cent. All these figures are calculated on the basic premise that the excretion of urea is unchanged. Obviously, the reabsorption of urea must be considerably less if the glomerular

filtrate is reduced to one-half and the flow of urine is maintained at the same level as when both kidneys were functioning. In the latter event the concentration is approximately 100 times, in the former only 60-70 times and thus the differences in the concentration will vary considerably in the two instances.

From the arguments in the foregoing it is seen that several possibilities exist for compensating the loss of one kidney so that individuals with only one kidney can manage as well as those who have two. A special problem is: which is the cause of one kidney functioning more effectively when it is alone? It must receive some sort of indication that it must take over all the work formerly carried out by both. This possibly takes place as follows. When the kidney has functioned for a very short time at the same rate as previously, disturbances arise in the form of a rise in the non protein nitrogen, changes in the water and sodium chloride balance, etc. The kidney's usual response to these changes in the normal balance is that of increased function. The non protein nitrogen, etc. then return almost to normal, although a residual small change is left as a warning signal to the kidney that its work must be continued on the same level. In the case of non protein nitrogen, the increase is presumably so small that in the single case it lies within the normal limits and cannot easily be discovered. It could of course be determined by examination of a large series of nephrectomized individuals in which the mean would show a difference against the normal.

An objection can obviously be made to the hypotheses in the foregoing, namely that the conditions were only discussed with reference to urea. Urea is, *per se*, non-toxic and does not give rise to uraemia. Nevertheless, the concentration of urea has a parallel course with the changes in renal function and if this is impaired, the urea nitrogen in the blood rises. Since we do not know which substance — or substances — cause the clinical picture of renal insufficiency, we must study some known substance. Urea is well suited to this purpose, particularly since the manner

of its excretion is more complicated than that of substances excreted through secretion or filtration or both. With a decrease in renal function, the conditions of excretion and reabsorption are naturally changed in respect of all the substances. For several of them, however, these changes take place easily owing to the extent of variation in the tubular cells. Urea occupies a unique position which is further emphasized by its relation to the excretion of water, which is one of the most important functions of the kidney.

Renal function following nephrectomy must now be studied with these aspects in mind. Animal experiments have been carried out — as mentioned earlier — but they are hard to interpret and partly contradict each other. Only a few cases are described in which renal functional tests following nephrectomy have been made in man. The present writer therefore attempted to make a special study of this problem.

## Cases with One Functioning Kidney

The present writer was able to examine 19 persons with one functioning kidney. Nephrectomy had been performed in 14 cases before the investigation and intravenous urography revealed one functioning kidney in the remaining five cases. The following data are given in tabular form (Table XXXIX): diagnosis, age of the patient at the time of the examination, figures for the inulin and diodrast clearances and the effective renal blood flow. The distribution according to age and sex is also found in this table as well as in the survey in Table XI (v. p. 95). It is seen from Table XXXIX that the material can be divided into two groups, i.e. those nephrectomized within a few years before the examination was performed and those operated on 11-22 years previously. Only one patient in the former group was over 30 years of age before the operation, whereas in the latter group only three of the seven cases were under the age of 30 when the operation was performed. The patients in the former group were therefore usually younger at the time of the examina-

Table XXXIX  
Survey of the writer's cases with one functioning kidney. Arranged according to the age,  
when the operation was performed.

D i a g n o s i s	Age at the examination, years	Time after the operation	Inulin clearance	Diodrast clearance	Effect. renal blood flow
Hydronephrosis*	24	16 years	75	360	720
Renal tuberculosis	40	23 "	81	215	379
Renal tuberculosis	37	17 "	99	410	684
Hydronephrosis	24	1 year	91	367	749
Hypoplasia, one funct. kidney	28	3 years	108	379	574
Rupture of the kidney	30.5	2.5 "	65	245	430
Lithiasis + atrophica renis	31	2 "	85	278	534
Renal tuberculosis	29	14 days	88	347	598
Nephritis apostenatosa	35.5	5.5 years	80	337	591
Renal tuberculosis	45	14 years	75	365	676
Pyelitis chron. + pyonephrosis	49	18 "	68	259	498
Renal tuberculosis	35	8 days	59	241	395
Hypernephroma	64	11 years	63	191	342
Cystopyelitis + calculus	72	16 "	67	211	335
Hypoplasia renis	20	8 days before	84	335	598
Renal tuberculosis	50	36 " "	87	243	343
Hydronephrosis	52	7 months "	79	308	642
Aplasia renis?	36	Not operated	71	220	407
Renal tumor, one funct. kidney	58	Not operated	46	204	376

\* v. page 214



tion, the mean age being 29.3 years, whereas the mean age for the latter group was 47.4 years. It must also be mentioned that none of the operated patients showed any symptoms from the remaining kidney in the form of proteinuria, elevation of the blood pressure, etc., with the exception of one patient in the latter group who had been nephrectomized 16 years earlier for congenital hydronephrosis (the case marked with an asterisk in Table XXXIX). It was obviously not possible in this case to exclude the possibility of lesions in the remaining kidney in spite of the fact that the clearance figures were of the same order of magnitude as in the other two patients in this group, who were also operated on before the age of 30 years. As will be shown later there is a difference which may be due to age. We shall, however, discuss the whole material before going into the influence of age.

The results of the tests in this group with one functioning kidney are seen summarized in Tables L and LI, which also

Table L

Comparison between normal cases and cases with one functioning kidney in regard to different tests.

Determination	Normal cases		Cases with one functioning kidney	
	Number	$M \pm s(M)$	Number	$M \pm s(M)$
Inulin clearance .....	56	$122.2 \pm 1.8$	19	$77.4 \pm 3.4$
Diodrast clearance .....	53	$444.2 \pm 5.7$	19	$290.3 \pm 16.1$
Filtration fraction .....	53	$0.275 \pm 0.004$	19	$0.274 \pm 0.011$
Creatinine clearance .....	21	$140.9 \pm 5.2$	9	79.0
Urea clearance .....	21	$80.3 \pm 3.9$	9	59.4
Effect. renal blood flow ....	53	$818.9 \pm 10.6$	19	$519.5 \pm 32.2$
Hæmatocrit .....	56	$45.7 \pm 0.5$	19	$43.4 \pm 1.3$
Proteinuria .....	—	—	19	$0.061 \pm 0.023$
Blood pressure { systolic ....	56	$130.4 \pm 1.0$	19	$141.6 \pm 4.9$
	56	$76.3 \pm 0.6$	19	$85.0 \pm 3.4$
Urea nitrogen, mg % .....	24	$17.7 \pm 0.8$	8	20.4

gives the normal figures and the differences. The differences were statistically significant for the inulin and diodrast clearances and for the effective renal blood flow. The filtration fraction, however, was the same in the nephrectomized as in the normal individuals (nor was there any difference in the hæmatocrit figure which was hardly to be expected). Both the systolic and diastolic blood pressure were somewhat higher for this group than for the normal material, but the difference was statistically probable for the latter only, calculated for both sexes together. It is naturally impossible to judge whether this elevation of the blood pressure was directly due to the nephrectomy, since 42 per cent of the patients were over the age of 40. It is obviously more probable that this small rise was due to the fact that so many of these patients were at an age when arteriosclerosis begins to become manifest, often with an elevation of the blood pressure. The »age factor» cannot, nevertheless, be responsible for the decrease in the inulin and diodrast clearances,

Table LI

Differences between normal cases and cases with one functioning kidney with regard to different tests. A minus difference signifies that the normal figure is larger, a plus difference signifies the contrary.

Determination	Normal cases — cases with one functioning kidney		
	Men	Women	Both sexes
Inulin clearance . . . . .	-48.4	39.8	-44.8 ± 3.8
Diodrast clearance . . . .	-157.2	147.4	-153.9 ± 17.1
Filtration fraction . . . .	0.013	+ 0.011	-0.001 ± 0.012
Creatinine clearance . . .	33.7	-73.6	-61.9
Urea clearance . . . . .	-21.6	16.8	20.9
Effect. renal blood flow .	-300.3	273.1	-299.4 ± 33.9
Hæmatoerit . . . . .	-0.8	2.2	-2.3 ± 1.4
Blood pressure {	systolic	+19.5	+11.2 ± 5.0
{	diastolic	+13.5	+8.8 ± 3.5
Urea nitrogen, mg % ..	+0.6	+3.1	+2.7

since this was present in practically each individual irrespective of age. Determination of the urea nitrogen was only performed in eight of the cases and the standard error of the difference could not therefore be calculated. This was also the case for the urea and creatinine clearances. The mean figure for these determinations is nevertheless included in the table in order to give an idea of how these results agree with the results of the other tests.

As seen from the survey of the literature in the foregoing only a few cases have been examined with modern methods and clearance tests. Ellis and Weiss (1933) and Friedman et al. (1942) thus found that some patients showed normal renal function following nephrectomy, whereas Welsh et al. (1944) found impairment of function in their two patients although — at any rate during the 40-month period of observation — it was not below 70 per cent of the normal figure. Köhler's (1944) investigation is less convincing, since he fixed the normal limits for creatinine clearance between 70 and 200 ml per minute, thus a very wide margin. Of his 56 patients, only six had a clearance below 70 ml/min, i.e. pathological figures according to the author's standard. No information can be obtained from the forementioned investigation regarding the number of patients in whom renal function was permanently impaired following nephrectomy, since their clearance before the onset of renal disease was not determined. If, for example, a patient «normally» had a clearance of 200 ml/min and reached a figure of 150 ml/min after nephrectomy, this implies in actual fact a 25 per cent decrease in the total filtration and an increase with the same amount, if this is computed on a single kidney. Since 150 ml/min is well within the normal limit, it is rather difficult in this case to judge the situation without knowing the figure previous to the operation.

The present writer's investigation on 14 nephrectomized patients and five with one functioning kidney showed that renal function, judged by the inulin and diodrast clearances, was decreased in comparison to that of healthy individuals, and that only one patient showed an inulin clearance value

over 100 ml/min (i.e. 108 ml/min). It is obviously impossible to state whether the figures for this patient reached the pre-operative level since no renal functional tests were made prior to the renal disease. Moreover, the results of the investigation appear to indicate that the degree of compensation arising after unilateral nephrectomy depends on the age of the patient at the time of the intervention and possibly also on the age at the time of the examination.

Table LII

Differences between cases with one functioning kidney operated before and after 30 years of age.

Determination	< 30 years		> 30 years		Diff.
	Number	$M \pm \varepsilon(M)$	Number	$M \pm \varepsilon(M)$	$D \pm \varepsilon(D)$
Inulin clearance.....	10	$85.6 \pm 4.6$	9	$68.3 \pm 4.9$	$17.3 \pm 6.7$
Diodrast clearance...	10	$327.3 \pm 22.1$	9	$249.1 \pm 23.3$	$78.2 \pm 32.1$
Filtration fraction...	10	$0.268 \pm 0.015$	9	$0.281 \pm 0.016$	$0.013 \pm 0.022$
Effect.ren.blood flow	10	$585.7 \pm 44.3$	9	$446.0 \pm 46.7$	$139.7 \pm 64.4$
Blood press. { diast.	10	$135.5 \pm 6.77$	9	$148.3 \pm 7.14$	$12.8 \pm 9.84$
	10	$83.0 \pm 4.68$	9	$87.2 \pm 4.94$	$4.2 \pm 6.8$

In order to elucidate this fact the material has been divided into two groups, i.e. those who were operated on before 30 years of age and those who were operated upon later. The former group consisted of nine individuals, the latter of ten. On account of the small material the standard errors are computed using the standard deviations for all the cases. It appears from Table LII that the value for the inulin clearance is somewhat higher in the patients operated on before 30 years of age and the difference was statistically probable. In respect of the other tests the figures are higher in this group although the differences according to the small material are not even probable.

If the present writer's figures are considered in the light of the discussion in the foregoing on the compensation mechanism following nephrectomy, the following facts are evident. The inulin and diodrast clearances are 65-67 per cent of the normal clearance, the creatinine clearance 56 per cent and the urea clearance 74 per cent. This means that the renal blood flow and the filtration rate have increased in the remaining kidney by approximately 30-35 per cent as compared to before the operation in this kidney, calculated on the diodrast and inulin clearances. If the increase in filtration is calculated in the same way on the creatinine clearance it is only 12 per cent. It is difficult to give the reason for this lower figure, but it is possibly due to random variation, the creatinine clearance determinations being considerably fewer. The increase of the urea clearance is, on the other hand, 50 per cent calculated on one kidney as before. If the difference is calculated on the total amount there is a decrease of 25 per cent which means that the urea level in the blood must be higher than before the operation if the reabsorption of urea were the same as before. As a matter of fact it should be 23.5 mg per cent, i.e. a third higher, if the figure obtained is correct. This fact will be discussed in more detail in General Discussion in the present paper (v. Chapter VIII). Urea nitrogen determinations were made in eight of the cases only. The mean figure was higher than in the normal material, the increase being approximately 11.5 per cent. According to the foregoing statements, this figure is too low and could be due simply to random variation, as the number of cases is rather small (the number was not sufficient for a computation of the standard error). The main cause, however, could be a lower reabsorption.

All these figures are in good agreement with the hypothesis in the foregoing regarding the compensation mechanism in such cases. It must, nevertheless, be borne in mind that the present determinations were carried out on patients of whom the majority had lived with only one kidney for a number of years and the figures obtained thus give no data

concerning the immediate compensation following nephrectomy. It is possible that there is a smaller increase in filtration rate and in renal blood flow and a greater increase in urea nitrogen level directly after such an intervention and that this gradually reaches the figures given here. This successive change would then be conditioned by the degree of hypertrophy in the remaining kidney with a gradual adaptation process towards normal conditions. In favour of this interpretation is the fact that function is more satisfactory — or perhaps it should be said that filtration and renal blood flow more nearly approach normal figures — if nephrectomy is performed at an early age when the possibilities of hypertrophy are greater than in later years.

As pointed out in the foregoing, the increase in the inulin and diodrast clearances was the same, i.e. approximately 30-35 per cent of the figure for one kidney. That the increase took place in the same proportion is also shown by the fact that the filtration fraction figure was the same in these patients as in normal individuals. An increase in the filtration fraction could otherwise be thought to depend on a change in the tonus of the efferent arterioles. Were this the case, however, the relation between the inulin and diodrast clearances would be affected and approach a higher level.

## CHAPTER VIII.

### GENERAL DISCUSSION

The results of the tests used have already been discussed in connexion with the diseases studied in the present paper, i.e. acute and chronic nephritis and essential hypertension. The primary aim of the present chapter is to compare the pictures in the various diseases and to analyze the results of the different tests and their correlation in particular. Finally, the way in which the kidneys work when part of them is eliminated will be touched upon, chiefly because different authors have expressed different opinions in this respect. In other words, the discussion here will be on a broader basis than before when the separate diseases were discussed.

The results of the functional tests in the various renal diseases can be summarized as follows:

1. *Acute nephritis*: low filtration fraction as an expression of a proportionately greater decrease in glomerular filtration than in »renal blood flow». The greater the injury to the kidney the more pronounced is this change (determined by inulin clearance, v. fig 7, p. 138).

2. *Essential hypertension*: high filtration fraction as a result of a proportionately greater decrease in the »renal blood flow» than in glomerular filtration.

This functional change is nevertheless not always present in cases of essential hypertension in this material, contrary to the conditions in series of other authors (e.g. Goldring and Chasis) but it is found in the majority of cases and it seems to be more pronounced the older the patient (v. fig 11 p. 196).

3. *Chronic nephritis*: although the filtration fraction lies within normal limits for this group as a whole, there is a distinct tendency with regard to the prognosis: with decreased function and a high filtration fraction the prognosis is bad, whereas with a low filtration fraction it is better. This latter fact is in agreement with the conditions in acute nephritis where the possibilities for an improvement in function are also obviously better than in progressive chronic nephritis. In the latter group only successive deterioration can be expected. The different results of the functional tests in these two groups, i.e. those with a bad and a better prognosis respectively, could reasonably be correlated to different localizations of the injury.

Of the forementioned types of disease, group 1 constitutes one extreme type and group 2 the opposite extreme, whereas group 3 occupies an intermediate position, changes of the type in group 2 predominating at times and those of group 1 at others.

Before continuing, it can be recalled that the filtration fraction is determined by dividing the inulin clearance by the diodrast clearance. The »renal blood flow» is obtained by taking into consideration the plasma content of the blood, i.e. by using the haematocrit figure. Under conditions that are otherwise equal, decreased filtration is naturally the expression of decreased blood flow. If, however, filtration is lowered by, for example, a decreased permeability in the glomerular membrane, it is no longer a question of unchanged conditions. Obviously decreased filtration in such a case is not necessarily a result of decreased blood flow.

What anatomical or functional changes in the nephron give rise to these different types of functional changes? In order to answer this question, the various possibilities must be considered.

As regards filtration, a decrease can depend on 1) a change in the permeability of the glomerular membrane but also on 2) circulatory disturbances of various kinds.

When considering the *permeability of the glomerular membrane*, it must first be borne in mind that the forementioned



diseases — at any rate at times — are accompanied by proteinuria. This implies that larger molecules can pass through the membrane than under normal conditions. If the membrane became consistently more porous, an increase in filtration should occur simultaneously. If the filtration does not increase, this must either depend on a considerable decrease in the filtration pressure in the glomeruli or on the fact that while a number of pores are enlarged, others are simultaneously occluded so that the total number of pores becomes less. With regard to the latter possibility, it can be recalled that proteinuria can arise very rapidly and also disappear rapidly. No very great increase in porosity appears to be required since in orthostatic proteinuria there is increased permeability of a transient nature, which is considered to depend on moderate stasis. An increase in porosity is obviously also a requisite for the penetration of a large number of red blood corpuscles.

If filtration is considerably decreased at the same time that proteinuria occurs, this can — as mentioned earlier — depend on the fact that certain parts of the glomerular membrane have ceased to function. A rapid change of this kind can be envisaged on the grounds of a swelling of the cells in the membrane only. When we are faced with more pronounced and less rapidly occurring changes in the glomeruli in the form of endothelial proliferation or sclerosis, there is no difficulty in envisaging decreased permeability with a smaller number of open pores but an increase in size of those pores remaining open, thus allowing the passage of the protein molecules. The forementioned swelling of the cells is more hypothetical as is also the increased size of the pores.

It can also be postulated that filtration decreases owing to *circulatory disturbances* despite an increase in the permeability of the membrane. It must anyhow be recalled that increased permeability presumably characterizes the majority of the renal vessels, to judge by the oedema of the renal tissue occurring in acute nephritis. This oedema should *per se* be a circulatory impediment. The blood vessels

are subjected to increased pressure from the surrounding tissues and therefore become somewhat compressed. Excessive oedema can practically inhibit circulation. This increased obstacle to circulation is counteracted to some extent by the elevation in blood pressure in acute nephritis. This elevation in the blood pressure should, *ipso facto*, give rise to increased filtration but the oedema can prevent any such effect.

Furthermore, the resistance to the flow can be brought about in another way in acute nephritis. A spasm in the afferent arterioles should decrease the filtration and a dilatation of the efferent arterioles should have the same effect. The latter mechanism should cause a decrease in the filtration fraction whereas the former need not affect it.

1. The changes in *acute nephritis* can nevertheless — at least in part — be of a functional character. The condition has a rapid onset and can be so transient as to indicate that functional changes are responsible to a considerable extent. Thus, if filtration decreases owing to circulatory changes, the following possibilities must be envisaged:

I. changes in the blood pressure,

II. a »by-pass» mechanism according to the theory of Trueta et al. or

III. changes in the resistance to the flow, i.e. changes of tonus in the efferent and afferent arterioles.

The *elevation in the blood pressure* — which is nevertheless usually fairly moderate in acute nephritis — has been touched on in the foregoing. It was pointed out that this can to a great extent be counteracted by the pressure of the surrounding tissues. In other words, it is impossible to state exactly how much of the elevated pressure is transferred to the glomeruli. An elevated blood pressure should naturally *per se* increase filtration, but if the pressure of the tissue is increased there can be a decrease in pressure in the glomeruli and a resulting decrease in filtration. It is scarcely possible that this could be the case.

A discussion of the »by pass» *mechanism* according to Trueta et al. (v. Chapter I) may be indicated in this con-

nexion. This theory implies that part of the blood in the kidneys is «shunted» via the juxta-medullary glomeruli and their efferent arterioles past the cortex and is emptied through the vasa recta into the medullary venous system. In other words part of the blood does not pass through the excretory tissue in the cortex, although the filtration process can take place on a normal scale. This is the direct opposite of the course in acute nephritis, when the renal blood flow and tubular function are less affected than glomerular filtration. The forementioned theory cannot therefore be considered valid.

It therefore remains to discuss whether *tonus changes in the afferent or efferent arterioles* can explain the results of the functional tests.

There are several possibilities, i.e. either an increase in the tonus of the afferent or efferent arterioles or a decreased tonus with resulting dilatation of either or both of these vessels. In respect of the filtration process this must — according to ordinary haemodynamic laws — adjust itself so that an increase in the afferent tonus and a decrease in the efferent tonus both give rise to a decrease in the glomerular pressure and of filtration, whereas the opposite — increased filtration — occurs with a decrease in the afferent and an increase in the efferent tonus. This process of reasoning obviously only holds good under otherwise similar conditions. i.e. constant blood pressure, unchanged colloid-osmotic pressure, etc. These factors are, however, very variable in the course of acute nephritis. This is particularly true — as mentioned in the foregoing — of the blood pressure, which is often elevated in the acute stage. At a later stage the colloid-osmotic pressure can also change, although the loss of proteins in this disease is only seldom of the order of magnitude that there are any very marked changes in the plasma proteins (as, for example, in nephrosis).

Moreover, it must be pointed out that in acute nephritis there are at times colloids in the filtrate and that the difference due to the colloid-osmotic pressure of the blood must then be smaller than under normal conditions.

The two existing possibilities for a decrease in filtration from a purely haemodynamic point of view are an increase in the tonus of the afferent arterioles or a decrease in that of the efferent arterioles. Since the renal blood flow is unchanged in mild cases and is decreased in more severe cases — although proportionately less than filtration — the only remaining possibility is an increased tonus in the afferent arterioles. That this is probably the case was demonstrated by Black, Platt et al. (1948) who determined the degree of this afferent renal arteriolar resistance. Their calculations, made with the help of L'ampour's (1941) formulae, showed that afferent resistance was considerably increased in their three patients during the hypertensive phase of acute nephritis but that when the blood pressure fell, the value for the resistance fell towards normal. In the opinion of the forementioned writers, a low filtration fraction is present only during the hypertensive phase of acute nephritis. This is nevertheless contradicted by the results obtained by the present writer (v. Chapter V, p. 138) i.e. the filtration fraction is low when renal function, assessed by inulin clearance, is very decreased (below 60 ml/min) irrespective of whether the blood pressure is, has been or has never been shown to be elevated. A low filtration fraction is found not only during the hypertensive phase but also later. This fact appears therefore to prove that decreased filtration is to a great extent dependent on other factors, e.g. decreased permeability of the glomerular membrane, as discussed in the foregoing.

To sum up the discussion it can be said that functional changes in acute nephritis probably depend on decreased permeability of the glomerular membrane but that a change in the renal arteriolar resistance presumably also plays a rôle. We must then take into consideration a decrease in the filtration pressure in the glomeruli owing to the increased pressure from the tissues and also — at any rate in a general elevation of the blood pressure — a constriction of the afferent arterioles.

2. The opposite type of functional change, with fairly

normal filtration and decreased effective renal blood flow, is characteristic of *essential hypertension*. The problem is considerably easier in this case than in the former group and the possible explanations fewer.

The importance of the general elevation in the blood pressure must first be discussed. It should, *ipso facto*, increase the filtration but scarcely the filtration fraction, if the elevated blood pressure has as much effect on the vessels peripherally of the glomeruli. The effect of the elevated blood pressure can, however, be counteracted by decreased permeability in the glomerular membrane or by increased tonus in the afferent arterioles so that filtration becomes normal. It is possible that both mechanisms play a rôle. In the early stage the probability of a change in the glomerular membrane is very small. In cases of longer duration there is more reason to expect sclerosis, particularly in cases of malignant hypertension.

The »by-pass» mechanism postulated by Trueta et al. (1946) can be thought to have some significance, particularly in the case of senile patients. In a preceding chapter (Chapter I) the changes in renal function in rising age, when arteriosclerosis becomes manifest, were discussed and this »by-pass» mechanism was advanced as a plausible explanation. It is possible in cases of »premature old age» manifest *inter alia* by arteriosclerosis with elevation of the blood pressure that this mechanism can operate and be partly responsible for the results of the functional tests in some hypertensive patients. In younger individuals with variable blood pressure it is more likely that there is another reason, i.e. a change in the tonus of the efferent arterioles.

In the discussion in the foregoing of the different possibilities to explain the results of the functional tests in acute nephritis, mention was made of the changes to which an increase or decrease in the tonus of the afferent or efferent arterioles could give rise. It transpired that the only change in the tonus of the arterioles that could cause a functional change such as that found in essential hypertension is an increased tonus or spasm in the efferent arterioles. The

supposition of such a change would explain the lower renal blood flow and — despite it — the normal or practically normal filtration in mild cases and the proportionately smaller decrease in filtration in more severe cases. This explanation, i.e. increased tonus in the efferent arterioles, is that generally accepted by all earlier workers who have discussed the reason for the results of functional tests in hypertensive patients. This discussion applies to the assumption that diodrast clearance is always an expression of the effective renal blood flow. If, however, the tubules are damaged and their secretory power in respect of diodrast is diminished, this discussion is no longer valid. A decrease in »renal blood flow» then naturally depends on this fact and the disproportionality in inulin and diodrast clearances is only due to more advanced tubular than glomerular damage. As a matter of fact determinations of diodrast  $T_m$  in essential hypertension have shown that a tubular damage also contributes to the decrease in »renal blood flow» in this disease.

Without entering into any discussion of the pathogenesis, either humoral or neurogenic, of essential hypertension, it can be recalled that after injection of hypertensin in a normal human subject, Page (1941) noted an increase of 40 mm Hg in the general blood pressure with a simultaneous decrease of 50 per cent in the renal blood flow without any increase in filtration. This obviously gives no information regarding the specific nature of the underlying change in the kidney other than that it is functional. The similarity to conditions in essential hypertension can indicate that the mechanism is the same in both cases. It can nevertheless in some instances be functional and reversible (initial hypertension with variable blood pressure) in other more advanced cases purely organically conditioned by sclerosis in the relevant vessels (advanced hypertension with stabilized pressure). A vicious circle can very well be envisaged and appears moreover as probable.

To sum up, it can be stated that the changes in renal function in essential hypertension, which are the exact opposite of those found in acute nephritis, can be explained

by an increased tonus in the efferent arterioles — and possibly even in the afferent arterioles — but that the »by-pass» mechanism postulated by Trueta et al. can also be contributory, at any rate in more advanced cases. In both instances the result is nevertheless a decreased blood flow to the tubular excretory tissue. The changes can also, at any rate partly, be explained by a more extensive tubular than glomerular damage.

3. In *chronic nephritis* functional changes corresponding to those in acute nephritis are found at times but at others those characteristic of essential hypertension. The presence of one or the other type is obviously correlated — as pointed out previously — to whether there is progressive chronic nephritis or an exacerbation of imperfectly healed acute nephritis. There is no reason to assume that any specific change arises in chronic nephritis. The same factors are probably contributory as in the two main types described in the foregoing, although it should be emphasized that the conditions of permeability in the glomerular membrane probably play a larger rôle in chronic nephritis.

This functional test can thus be classified as a topographical diagnostic one in so far as changes of the nature found in essential hypertension chiefly point to a change in the vascular system distal of the glomeruli and possibly of the tubular apparatus as well. On the other hand, functional changes in the opposite direction presumably indicate changes proximally of or in the glomeruli themselves or changes that are manifest in some other way with regard to glomerular function (an increase in intrarenal pressure).

It can also be emphasized that those types of disease that are associated with proteinuria and haematuria must be correlated to an increase in the permeability of the glomerular membrane which at the same time is less permeable in certain other parts. Were this not the case, proteinuria would be accompanied by increased filtration on the grounds described in the foregoing.

In dealing with the correlation of the various functional tests in impaired renal function and their value in assessing it, the present writer based his conclusions on the correlation calculations of results of the tests used in the investigation. In making the calculations, the entire material was assembled into one group, irrespective of whether the individuals were diseased or healthy. The correlation was then calculated between the following:

- a) inulin and creatinine clearance,
- b) inulin and urea clearance,
- c) inulin clearance and the concentration test and
- d) diodrast clearance and the concentration test.

In addition, a computation was made in order to ascertain whether the filtration rate, determined by inulin clearance, had any connexion with the increase in the non protein nitrogen and urea nitrogen above the borderline figures given in the literature, i.e. 40 mg per cent and 20 mg per cent respectively.

Before these correlation calculations are discussed, the following theoretical aspects must be pointed out. If, for example, a test is the expression of the activity in the kidney of certain cells, this will naturally vary more or less depending on various factors. If another test is an expression of the activity of the same cells, it will presumably be affected by the same factors as the former test, in which case both tests will be more or less intimately correlated. If they are an expression of the same kind of activity in the cells, the correlation will be absolute. If, on the other hand, they are an expression of different aspects of the cellular function, they will probably only show moderate correlation. Finally, if two tests refer to the activity of different groups of cells, they may be only slightly or not at all correlated.

When we are dealing with individuals whose kidneys are impaired, this can be still more evident. By including both diseased and healthy individuals, greater variation is obtained and thereby greater possibilities for correlation. If two tests refer to one and the same group of cells, they will both be



affected when injuries are present in the cells. This means that any possible correlation will be more distinct. If, on the other hand, two tests refer to separate groups of cells, there are greater prospects that they will be shown to be independent. They will, however, be correlated in more extensive injuries which affect both groups of cells. These indications, which are fairly self-evident, are given in order to justify the amalgamation of results from diseased and healthy individuals. This procedure gives a larger material and also affords greater possibilities of revealing correlations. Their significance must obviously be discussed and possibly subjected to further analysis.

A few correlations are first given in the case of normal individuals only, although the material is then relatively small. The results of the calculations of the correlation between the inulin clearance and the creatinine and urea clearances are seen from the survey below.

Table LIII

Coefficient of correlation,  $r$ , and its standard error,  $\varepsilon(r)$ , for normal cases in regard to inulin clearance—creatinine clearance and inulin clearance—urea clearance respectively.

	Number of cases	$r$	$\varepsilon(r)$
Inulin clearance — creatinine clearance	21	$0.38 \pm 0.19$	
Inulin clearance — urea clearance	23	$0.29 \pm 0.19$	

The material is unfortunately small, only 21 and 23 cases respectively, on whom all these tests were performed simultaneously. It is seen that the correlation found is not significant, but the figures nevertheless give the impression that a moderate correlation exists. The calculations were made chiefly to justify the procedure in not separating the material into groups. The figures distinctly show that the groups would then be too small and the mean figures too large.

The results obtained from the total material will now be discussed.

Table LIV

Coefficient of correlation,  $r$ , and its standard error,  $\varepsilon(r)$ , for different tests. The standard deviation,  $\sigma$ , for the 2nd test of the correlated pair is also given, as well as the standard deviation,  $\sigma_a$ , around the regression line.

Correlation between	Number	$r \pm \varepsilon(r)$	$\sigma_a$	$\sigma$
Inulin clearance — Urea clearance	205	$0.818 \pm 0.023$	15.2	26.4
Inulin clearance — Creatinine clearance .....	157	$0.893 \pm 0.016$	18.9	42.0
Inulin clearance — Water tolerance test.....	143	$0.453 \pm 0.066$	0.005	0.006
Diodrast clearance — Water tolerance test.....	143	$0.036 \pm 0.084$	0.006	0.006

Although there has been much discussion regarding *inulin* or *creatinine clearance* as an exact measure of glomerular filtration, all workers are nevertheless agreed that when a change in it takes place it is reflected to about the same extent in these two clearances (v. Chapter I). In the writer's calculations of their correlation in 157 patients on whom these clearance tests were performed simultaneously, the correlation coefficient was  $0.893 \pm 0.016$ , thus showing that a strong correlation exists. This is also seen from Table LIV and fig 15.

The regression for creatinine clearance in relation to the inulin clearance is shown by the curve. It is found to be in agreement with a straight line and when the inulin clearance increases by 10 units, the creatinine clearance increases by 10.8 units. In other words, it is evident that the two tests run parallel to each other. The calculated standard deviation around the line of regression is 19 units, compared with the standard deviation in the entire material, which is 42 units.

*Urea clearance* has long been used as a measure of renal function. Several writers consider this test to be superior to filtration determinations with inulin or creatinine on the grounds that urea clearance is not only a function of the

rate of glomerular filtration but also of the functional ability of the tubules (v. Chapter I, Urea clearance). In other words urea clearance not only registers a partial function of the kidney but also throws more light on the activity of the entire nephron.

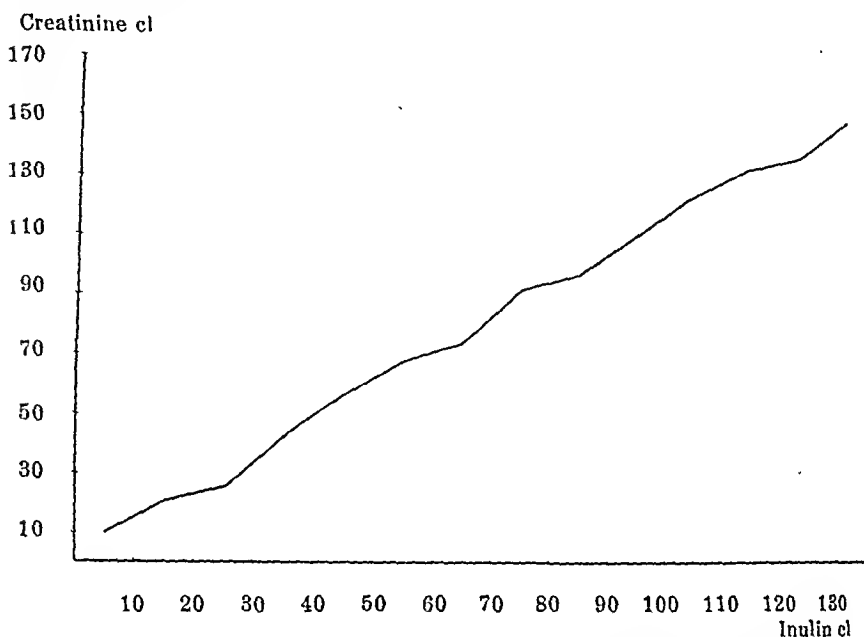


Fig 15. Diagram showing the regression curve of creatinine clearance in relation to decreasing values for inulin clearance. Both clearances expressed in ml/min.

Nevertheless, as already pointed out (v. Chapter VII, One Functioning Kidney) and as will be discussed subsequently, the resorption of urea from the tubules is probably chiefly a passive process correlated to the degree of water reabsorption and thus possibly as well to the size of the concentration difference between the tubular urine and the surrounding tissue and to the speed with which the fluid passes the tubules, etc. Under such conditions the reabsorption of urea can scarcely be a measure of the functional ability of the tubules, but is largely a secondary consequence of water reabsorption through the tubules, which can be calculated exactly by a comparison between the quantity of water filtered and excreted.

From these aspects it was of interest to investigate how the urea and the inulin clearances were correlated. In the statistical calculation (cfr. Table LIV) made on 205 patients with simultaneous inulin and urea clearance, the correlation coefficient and its standard error were  $0.818 \pm 0.023$ , thus a distinctly significant correlation. In fig 16 there is

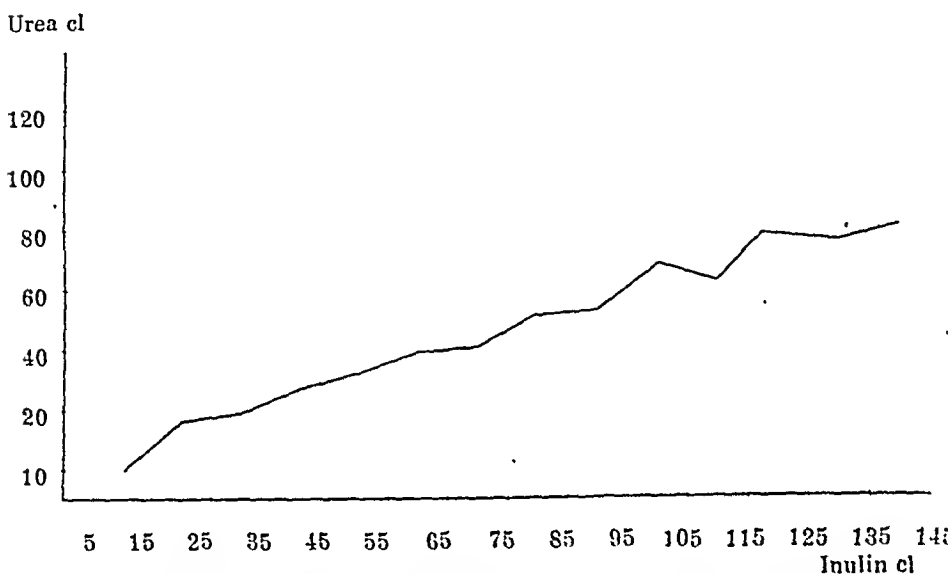


Fig 16. Diagram showing the regression curve of urea clearance in relation to decreasing values for inulin clearance. The latter expressed in ml/min, urea clearance in per cent of a normal value (100 %).

also a curve for the urea clearance, drawn on similar premises as those used for the creatinine clearance. It is seen that the curve of regression is in a straight line. It is found that when the inulin clearance increases by 10 ml, the urea clearance increases by 6 ml. That the increase is smaller for the latter is due to the fact that the standard deviation is considerably lower for urea, i.e. 26 ml, compared with 42 ml for creatinine. The calculated variation around the line of regression is 15 ml and is thus somewhat smaller than for the creatinine clearance.

Opinions have differed considerably on the value of the *water test* as a measure of renal function (v. Chapter I). The majority of workers are, however, in agreement on one

point, namely that the dilution test does not give reliable information on the condition of the kidney since extra-renal factors often influence it. On the other hand the maximal concentration capacity test is considered by many to be the best existing measure of renal functional ability.

In order to investigate in the present material how the maximal concentration capacity is correlated to renal function determined by inulin and diodrast clearance, a correlation calculation was made between the maximal concentration and these clearance values separately, based on a material of 143 individuals. It is seen from Table LIV that the correlation coefficient and its standard error in the calculation of the inulin clearance and the water test was  $0.453 \pm 0.066$  and can thus be designated as moderately strong. No significant correlation could be shown between the diodrast clearance and the water test, the corresponding figure being  $0.036 \pm 0.084$ .

The latter result can possibly at first sight appear somewhat puzzling, since both the water reabsorption and the excretion of diodrast are mainly effected by tubular activity. This incongruity can depend on several factors. Water reabsorption is largely governed by hormonal factors which — if they are not without effect — should nevertheless play a considerably smaller rôle in the case of diodrast. Secondly, in one instance we are dealing with reabsorption and in the other with excretion and these two functions, although they both take place through the tubules, do not in the vast majority of cases run a parallel course. Furthermore, both processes — at least to the greatest extent — are reflected in different parts of the tubules. It can also be pointed out that the water test — or rather the concentration test — is a measure of the maximal working capacity of the tubules, whereas this is not the case when the diodrast clearance is determined.

Fig 17 shows the curve for the maximal concentration, calculated from the mean figures at rising inulin clearances. It is seen from this regression curve that the rise is relatively slight. If the inulin clearance rises by 10 units, the specific

gravity rises by 0.0006. The variations in specific gravity are not large, the standard deviation amounting to only  $\pm 0.006$ .

A collocation was also made to determine the relation between the non protein nitrogen and the urea nitrogen

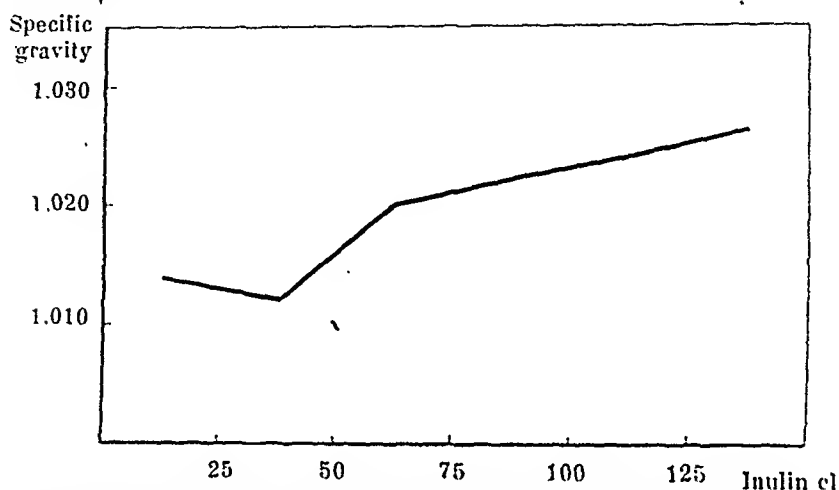


Fig 17. Diagram showing the regression curve of the maximal concentration in relation to decreasing inulin clearance (in ml/min).

respectively and the filtration rate. The results are seen in fig 18. The curve for non protein nitrogen is based on the determinations on 62 patients and that for urea nitrogen on 231 patients. To facilitate a comparison the same classification was used for both. Owing to the larger material in the case of urea nitrogen smaller groups could otherwise have been used. It was found that the curves started to rise above normal only when the inulin clearance fell below 70 ml/min for urea nitrogen and below 50 for non protein nitrogen, if the customary borderlines for the normal values, i.e. 20 mg per cent for urea nitrogen and 40 mg per cent for non protein nitrogen, were used. These levels are marked on the diagram. The upper borderlines obtained in the present writer's normal material are also marked, i.e. 29 mg per cent for urea nitrogen and 46 mg per cent for non protein nitrogen.<sup>1</sup>

<sup>1</sup> The latter figure for the upper limit for normal values was obtained from as yet unpublished determinations made by Dahlberg and Josephson.

If the latter borderlines are used, the curve for urea nitrogen crosses this limit at a filtration rate of approximately 40 ml/min, and the non protein nitrogen curve crosses it at approximately 30. In other words the curve for urea nitrogen crosses these limits in both cases earlier

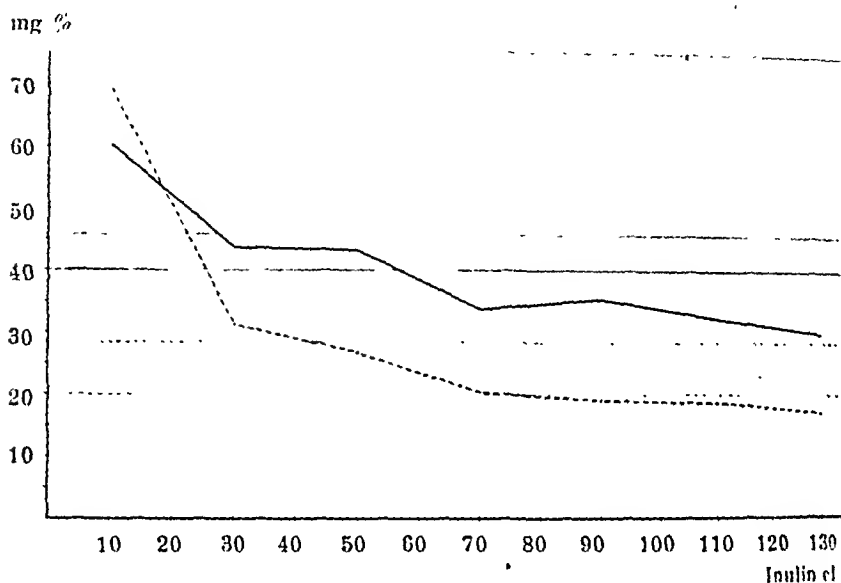


Fig 18. Diagram showing the relation between non protein nitrogen (thick line) or urea nitrogen (dotted line) and inulin clearance. The horizontal lines indicate the limits for normal values according to the literature (lower line) and to the writer's upper limits (3 $\sigma$ ). Dotted lines refer to urea nitrogen.

than does the curve for non protein nitrogen. This is interesting in itself. What is perhaps more important is that the curves start to rise above the normal borderlines only at such low inulin clearance values. Even if the number of determinations on which the urea nitrogen curve is based is not so large, it is nevertheless possible to state that azotæmia only starts to appear when renal function is reduced to far below 50 per cent of the normal, presumably when filtration has dropped to between 20-30 per cent. Both curves are calculated from the mean figures for groups with increasing levels of inulin clearance.

Recently, there has been some discussion regarding wheth-

er *creatinine* or *inulin clearance* is the more correct expression of the filtration rate. If small doses of creatinine are used, fairly considerable differences in the values are found but this difference is less apparent with larger doses. On what does this difference depend? The discussion of this problem is found in Chapter I. Without making any categorical statements it is nevertheless possible to discuss which test is most suitable from a clinical viewpoint.

It must first be recalled that the creatinine test is far simpler to perform both from the point of view of the patient and of the laboratory. A possible disadvantage is that creatinine is slightly more expensive but this need scarcely be a decisive factor. Under such conditions the only criterion is the degree to which the test is suitable for the diagnosis of disturbances in renal function.

The strong correlation demonstrated in the foregoing indicates that they both depend mainly on the same factor i.e. filtration. Actually, there is scarcely any difference of opinion in this respect. It is probable that creatinine is also excreted through the tubules although in a relatively insignificant proportion. Others are of the opinion that a small proportion of inulin is reabsorbed. Even if this were so, this fraction is of no significance, otherwise a correlation as strong as that found would scarcely become manifest.

The decisive factor is, as mentioned, the degree to which pathological changes in the kidney can be diagnosed with the use of these tests. The figures given earlier for the three diseases investigated here — acute and chronic nephritis and essential hypertension — show that the difference can not be great in this respect.

In order to throw more light on the problem and to make use of the entire material, information is given in the following of the number of cases falling outside the normal limit if this is denoted by  $2\sigma$  and  $3\sigma$  respectively below the mean figure, when the material for the various groups of diseases is amalgamated. With a borderline of  $2\sigma$ , the figure for the inulin clearance is then 64.8 per cent and for the creatinine clearance 52.2 per cent. The difference between these percent-



lages is not, however, significant (the difference is  $12.6 \pm 6.9$ ). If we compare the tests with a borderline of  $3\sigma$ , we obtain 50.3 per cent and 34.8 per cent respectively. The difference between these figures is not significant, not even statistically probable (it amounts to  $15.5 \pm 6.7$ ).

It is therefore possible that there are somewhat greater possibilities of judging a case with the use of inulin than of creatinine, but the difference is not large. The choice is therefore largely a matter of taste. The greater amount of work and of discomfort for the patient must be weighed against the small increase in accuracy obtained. The following point of view can therefore be justified. Creatinine clearance can be used as a routine method, in doubtful cases the investigation can be supplemented by inulin clearance.

The clinical importance of *urea clearance* will now be discussed.

As mentioned previously, inulin and creatinine clearances are both expressions of practically the same partial function, i.e. glomerular filtration, whereas urea is to a great extent reabsorbed in the tubules. This active or passive reabsorption varies considerably in relation to the urine flow. It was mentioned in the survey of the literature that Van Slyke et al. point out that urea clearance gives different figures on either side of a borderline of 2 ml/min. It is obvious that no very marked change occurs exactly at this borderline (v. the more detailed discussion in Chapter I). It can also be mentioned that the filtration rate can affect the reabsorption directly so that with a high filtration the rate of flow in the proximal tubules is greater than if the filtration is small. This should, *per se*, decrease the possibility of reabsorption. If a decrease in filtration is not combined with damage to the tubular apparatus, the urea clearance should — from this point of view — decrease more than inulin clearance with a decrease in filtration. Since it is evident that this is not the case, this factor cannot be entirely explanatory but there must exist other or contributory factors (e.g. decreased concentration of the tubular urine, v. the following). Shannon (1938) mentioned *en*

*passant* that the rate of flow can have some influence, but he did not discuss this point in any detail.

The importance of urea clearance in clinical practice, i.e. for assessing and diagnosing pathological cases, remains to be discussed. The number of cases falling outside the normal range of variation was therefore calculated for the whole material, as in the preceding cases. If this borderline is fixed at  $2\sigma$ , the figure is 35.9 per cent of 117 cases. If  $3\sigma$  is used, the corresponding figure is 12.8 per cent. Both figures are thus significant and considerably lower than for the inulin and creatinine clearances. This condition is found in calculations for all three diseases separately and is presumably correlated with the fact that urea clearance is compensated to some extent even if the filtration falls below the normal level. Urea clearance can therefore certainly be said to represent the result of the various partial functions of the kidney.

Volhard's *water test* with determination of the maximal concentration capacity was that test that gave least information concerning renal function in the present investigation.

It is not possible to express an opinion on the clinical value of the water test here, since it was relatively seldom used in the cases investigated. For this reason it was not discussed earlier. It can be recalled that many writers consider the maximal concentration capacity to be an extremely good measure of renal function. It is possible that this is the case if another method is used to determine the concentration capacity. The method used by the present writer, i.e. concentration following water tolerance test — thus a combined dilution and concentration test — is in any case far from the ideal.

Finally, when the question of the value of *diodrast clearance* as a renal functional test is to be judged, we return to the problem put forward in Chapter I. (The Problem) This will be discussed at this juncture.

It was seen in Chapters V, VI and VII that it is possible, by making creatinine or inulin and diodrast clearance tests simultaneously, to obtain considerably more accurate in-

formation both with regard to the diagnosis and the prognosis in different renal disorders than by making only one of these determinations. Since glomerular filtration and the »renal blood flow» do not change proportionately, but at times one and at times the other manifest greater changes, certain main types of functional changes can be indicated, corresponding to distinct clinical pictures. A filtration determination alone does not suffice to reveal the degree and the nature of the actual functional disturbance, nor does it provide the same prognostic possibilities as combined tests. Although the combined tests are more laborious technically than a filtration determination alone, the information thereby obtained is so considerable that it is necessary for accurate assessment of renal function.

The question of whether inulin, creatinine or urea clearance is preferable, as well as the value of Volhard's water test, non protein nitrogen and urea nitrogen determinations as renal functional tests were discussed earlier in this chapter.

The question »how far is it possible to judge renal function from clinical investigations alone?» has already been answered in the various sub-divisions of Chapters V, VI and VII.

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The third main question in this chapter, i.e. the working of the kidney in an impaired functional state, remains to be discussed. This problem is intimately connected with the conception of the kidney's reserve capacity, which was discussed earlier in connexion with renal function following nephrectomy. Two main conceptions were found to exist. According to one, this reserve capacity is due to the fact that a large number of nephrons in the healthy kidney are inactive and only start to function when necessary. The other explanation is that certain other changes take place in renal function to compensate the loss of parenchyma. The results of renal functional tests on nephrectomized patients indicate that the latter conception is the most probable.

These results are of importance for the subsequent discussion and will therefore be returned to later.

The greatest controversy in judging renal function in severe injuries to the kidney is whether inulin and creatinine clearance, which can both be considered as an expression of the volume of glomerular filtration, also constitute a measure of it in cases of advanced renal disease. As in the question of the reserve capacity, there are two different theories on this matter. Their principles are briefly the following.

One postulates that the reserve capacity of the kidney consists of resting nephrons. When renal injury is so extensive that renal insufficiency has started to develop, this can manifest itself in three different stages (Ekehorn 1938, 1946): 1) the initial stage, 2) the intermediate stage and 3) the final stage.

According to this theory, the initial stage is characterized by normal or hypernormal inulin and creatinine clearances and a considerably lower urea clearance.

In the final stage, on the other hand, the inulin, creatinine and urea clearances approach each other and are often similar, but all are definitely reduced.

In the intermediate stage there is a normal relative proportion between the urea, inulin and creatinine clearances, although they can all be considerably depressed.

To explain these three stages, the hypothesis is advanced that renal insufficiency is largely — or chiefly — due to the fact that initially the tubular epithelium allows an increased quantity of urea to rediffuse into the blood. With increasing renal injury this epithelium gradually becomes increasingly permeable not only to urea but also to creatinine and inulin. Owing to the rediffusion of the two last-mentioned substances, the values obtained for the inulin and creatinine clearances are too low compared with the actual filtration. These tests thus give an incomplete idea of renal function in severe impairment. Azotaemia in severe renal damage can therefore be regarded mainly as a manifestation of tubular insufficiency conditioned by uninhibited reabsorption.

According to the second hypothesis, there are no resting nephrons in the human kidney. In renal damage of various kinds the number of functioning nephrons is successively decreased, but those remaining act in principle in the same way as in the healthy kidney except that they take advantage of the existing possibilities for adaptation to increased function, necessary in view of the number of injured nephrons. The chief supporters of this theory are Smith and his school. They explain renal insufficiency as depending on the increasingly lowered filtration and consider it to be mainly a glomerular manifestation.

Without entering into a detailed critical examination of these theories, a few brief observations can be made in consideration of the results obtained in the present investigation.

Ekelhorn's classification into three different stages of renal insufficiency — initial, intermediate and final — was chiefly — if not altogether — founded on values published by Cambier (1934). The latter made creatinine and urea clearance tests on 10 healthy individuals and 73 with different renal disorders. Raised urea nitrogen values in the plasma were found in several of these patients, despite a normal — or in some cases hypernormal — filtration rate (determined by creatinine clearance). In many of them the urea clearance was also low compared with the high filtration. This was interpreted as dependent on the fact that an unusually large quantity of urea had been rediffused through the tubular walls. According to the theory put forward, more urea than normal is rediffused during the intermediate stage since the tubules are more severely damaged and even creatinine can no longer be retained in the tubular lumen but a part is also rediffused. The relation between the clearance values should approach the normal proportion. In the final stage the tubular lesions are maximal and urea and creatinine rediffuse to approximately the same degree and their respective clearances must therefore be almost identical.

Ekelhorn considered that he was able to demonstrate

the same conditions in regard to inulin and urea by means of a detailed study of a paper by Chasis and Smith (1938) dealing with the conditions of these clearances in healthy and nephritic individuals.

As regards Cambier's basic determinations, on which the whole theory is founded, it is a trifle strange that patients are so often encountered with no renal disease (cases of aeromegaly, diabetes, etc.) but with raised blood urea nitrogen. Amongst these are also patients with «hypernormal» filtration figures (according to creatinine clearance). It is possible that these figures are conditioned by endocrine factors. However this may be, such diseases are scarcely connected with renal disease. Cambier also found high filtration values in some diabetics. In the present writer's material of 12 such cases no high filtration values were found (cfr. Hogeman 1948).

In Chasis' and Smith's paper (1938) they made a survey of the urea and inulin clearances in normal and nephritic individuals. These writers demonstrated that the ratio of urea clearance to inulin clearance rises in both categories when the inulin U/P ratio falls. It is recalled that the U/P ratio is the concentration in the urine divided by the concentration in the plasma. If, in other words, these are similar,  $U/P = 1$  and the stronger the concentration of the tubular urine, the higher the quotient rises above 1. This ratio is obviously the same whether a volume of filtrate of, for example, 120 ml/min is concentrated to a final urine volume of 6 ml/min or a filtration of 20 ml/min gives a urine volume of 1 ml/min. In both instances the U/P ratio will equal 20.

It appears from the graphs of the forementioned writers that the same tendency to an increase in the ratio of the urea to the inulin clearance is found in normal individuals with a falling inulin U/P ratio as in nephritics. They therefore draw the conclusion that those nephrons that have remained capable of function in a progressive renal disease act, in principle, in the same manner as the nephrons in the healthy kidney. Their graph for normal individuals shows

the U/P ratio from 200 down to 6 and for the nephritic patients from slightly above 100 to 2. The reason that it was impossible in the case of normal individuals to obtain direct values for comparison with the nephritics with a U/P ratio below 6 is naturally that it was difficult to produce sufficiently high diuresis in the former to reach such a ratio (a diuresis of 20 ml/min is required). A reconstruction of the curve was made instead with the help of experiments on dogs carried out by Shannon (1938) since the conditions for urea reabsorption were found to be in agreement with those in man.

On the basis of the theory of increased tubular permeability in renal insufficiency, Ekelhorn (1946) demonstrated how Chasis and Smith's diagram could be interpreted in agreement with this theory regarding renal function in increasing damage to the kidney.

If these two theories are to be discussed in the light of the results achieved by the present writer, the following observations can be made.

1. In my material of nearly 300 cases no values approaching the high level of Cambier's series occurred. It has previously been pointed out that this can possibly be due to endocrine factors in certain of his cases. It is also possible that differences in the methods can play a rôle.

2. If the theory that renal insufficiency in the initial stage is caused by increased permeability for urea and thereby an increase in non protein nitrogen despite normal or »hypernormal« filtration were valid, it should have been possible to observe this in the present material in which a large number of the patients were at this stage. A study of fig 18 in which the urea nitrogen figure is given with decreasing filtration nevertheless shows that no rise whatsoever in urea nitrogen was manifest before the filtration rate — measured with inulin and/or creatinine clearance — fell far below 50 per cent of the normal.

3. We are aware that urea reabsorption is dependent on the degree of water reabsorption and thus on the degree of concentration of the glomerular filtrate, since urea clearance

varies with the filtration rate and with the urine flow (v. the foregoing). This is also illustrated by the conditions following nephrectomy, when the total filtration decreases but that of the remaining kidney increases compared with that before the intervention. That the output of urine in these cases is normal is conditioned by the fact that the water reabsorption and that of urea decreases compared to its previous size. These changes cannot be associated with a change in the actual permeability of the tubules. That the difference here between the urea and the inulin clearances was smaller than in the healthy individuals must be due to the fact that the glomerular filtrate is concentrated to a lesser degree in the case of the patients with diseased kidneys.

This fact must therefore be borne in mind when renal function is to be assessed by urea clearance in damaged kidneys. Particularly when the impairment is very pronounced, values relatively too high are obtained with urea clearance.

1. Figs. 15 and 16 also show that with impaired renal function the inulin, creatinine and urea clearances change proportionately to approximately the same degree until the filtration rate is very low. The present writer can only interpret this to indicate that the kidney handles these different substances in principle in the same way in healthy and in nephritic individuals. He thus shares the opinion of Chasis and Smith.

With very low filtration figures this proportionality is less pronounced. This can possibly be due to the fact that with severe renal damage the inulin and creatinine clearances do not express the true filtration rate but only a part of the respective substances rediffuses through the tubules. It must be borne in mind that in advanced renal disease the tubular lumen is enlarged and the rate of filtration considerably lowered. The rate of flow in the tubules is therefore much slower than normal and the time available for rediffusion or reabsorption is considerably longer.

It nevertheless appears unlikely that rediffusion of these substances takes place since their respective clearances fall



to practically the same degree. If there was an increase in the permeability of the tubular walls there should be some difference -- as pointed out by Chasis and Smith -- between creatinine, which is a low molecular substance and relatively easily diffusible, and inulin which is high molecular and has a diffusion coefficient of the order of magnitude found in molecules with a molecular weight of approximately 17000.

To sum up the writer's investigation of this matter, it can therefore be concluded that a decreased filtration rate with the accompanying lesser concentration of tubular urine must play a large and presumably decisive rôle in the convergence of the urea and inulin and urea and creatinine clearances respectively. No hypothetical and as yet unestablished increase in tubular permeability in increasing renal damage need be postulated in order to explain the conditions of these clearances in renal insufficiency.

## Summary

In a survey of the *literature on renal function* it is found that the modern methods used to test it have mainly been applied to normal individuals and that only a small number of determinations on pathological cases exist. This does not apply to essential hypertension concerning which several large investigations have been reported. It is emphasized that in respect of inulin clearance no large number of cases with acute or chronic nephritis has been examined.

The aim of the present investigation is therefore to establish the clinical importance of these types of clearance tests as far as is possible on the material available.

The clinical material consists of altogether 298 individuals (242 pathological cases and 56 healthy individuals). The former are distributed as follows: acute nephritis 89, chronic nephritis 48, essential hypertension 62, diabetes 24.<sup>1</sup> The other 19 patients had only one functioning kidney and are included in order to obtain an idea of the reserve capacity of the kidney.

The 56 healthy individuals between the ages of 13 and 46 years are used as a *normal control group*. The clearance values obtained for these normal individuals are in agreement with those usually given in the literature. An exception is the diodrast clearance for which the writer's values are lower, owing to the fact that a single intravenous injection of diodrast is given (v. p. 80). Somewhat lower values for urea clearance than those generally accepted are also found. This is presumably explained by the fact that —

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<sup>1</sup> An account of these latter cases is given in a separate paper in press (Acta med. Scandinav. 1918. Suppl. 216 b).

with the technique employed — the urea nitrogen values in the urine are relatively too low as compared with those in the plasma (v. p. 73).

In the performance of the tests certain facts are established, which may possibly be of general interest.

1. Different results are obtained with *varying brands of creatinine*. This is a source of error of considerable importance and implies that caution must be exercised in comparing the results obtained by different investigators.

2. It is demonstrated that in iodine (diodrast) determinations, the level of the *combustion temperature* and the time for combustion should be adjusted according to the amount of protein present in the different samples. The writer finds that 440° C is a suitable temperature for plasma and 300° C\* for urine.

3. As regards the technique in diodrast clearance tests, both continuous infusion and single injections have formerly been used. No detailed discussions of the reasons for the slightly different results obtained with these two methods are found in the literature. The writer discusses this problem (Chapter II, p. 78) and concludes that the fact that the *diodrast is bound to the plasma proteins* presumably plays the greatest rôle in the decreasing clearance values after a single administration.

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The different groups of diseases are dealt with as follows. The mean figures for the tests in the various diseases are compared with the mean figures for the normal individuals. A calculation is then made of the number of cases falling outside the normal limits of variation. Finally, it is determined if a case outside the normal limits of variation of one test also lies outside the normal zone of the other tests or not.

4. It has previously been shown that the filtration fraction is low in *acute nephritis*. The new results obtained here are mainly the following:

In acute nephritis, *filtration* — as determined by *inulin clearance* — is decreased in a relatively large number of

cases. This has been established earlier in smaller series. In agreement with the findings of Hilden in his 24 cases a simultaneous but relatively less marked decrease in the *diodrast clearance* is also found in the present material, resulting in a *low filtration fraction*. The writer is thus able to confirm earlier observations. He has found further that if the filtration rate falls below 70 ml/min, there is a decrease in the filtration fraction in practically every case. The diagnosis: *acute nephritis* may thus be confirmed and a certain basis for the prospective duration is obtained.

5 The writer shows that if, at the time of the investigation, the filtration is below 70 ml/min the patients do not as a rule recover within three months irrespective of the degree of hypertension, oedema, etc. In other words, a patient with decreased filtration requires longer hospitalization. This fact nevertheless does not provide us with any prognostic information as to subsequent recovery or the development of chronic nephritis.

6. As regards *chronic nephritis* the writer finds no change in the filtration fraction when comparing these cases as a group with the normal material. A closer analysis nevertheless reveals that the prognosis was poor in patients with decreased filtration and a high filtration fraction, since 13 of 15 patients died, all except three within a year. On the other hand the prognosis was considerably more favourable for many patients with decreased filtration but a low filtration fraction, since an improvement was found on a later repetition of the tests. In the latter cases it must have been the question of an exacerbation of an imperfectly healed glomerulonephritis. The writer also finds that cases with pronounced retinal changes and raised non protein nitrogen showed more pronounced impairment of renal function compared to cases grouped according to the presence or absence of other symptoms. The cases with elevation of the diastolic blood pressure thus manifested no more severe changes than those with normal diastolic pressure.

7. Contrary to general opinion, the writer finds that in his cases of *essential hypertension* a large number (approx-

imately 25 per cent) have no raised filtration fraction although the clinical picture is otherwise typical. The filtration fraction thus gives no definite guidance for the prognosis in this condition. On the other hand, it shows a definite tendency to rise with increasing age. With an inulin clearance below 75 ml/min, the prognosis is nevertheless poorer than with high clearance figures although no more than 20 per cent of deaths were due to uræmia. Furthermore, the writer finds no correlation between the results of the clearance tests and the level of the diastolic blood pressure. As could be expected, the cases with severe retinal changes showed particularly poor results in these tests. It may be mentioned that in another publication the author has analyzed the question of retinal changes in diabetic nephropathy (Acta med. Scandinav. Suppl. 216 b).

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8. In cases with *one functioning kidney* the writer finds increased function in the remaining kidney but in some ways decreased function as compared with normal individuals. After a theoretical discussion of the possible explanations of how one kidney can perform the work of two, the writer reaches the following conclusion. The urea concentration in the blood increases and there is also a certain increase in the filtration rate in the remaining kidney and a decrease in the reabsorption of urea. These different factors are responsible to a greater or lesser degree depending on the age of the patient: the younger the patient at the time when one kidney is forced to do the work of two, the greater are the chances of hypertrophy and of a return towards normal conditions.

9. The patho-physiological background of the functional changes in the different diseases investigated is discussed.

In *acute nephritis* the writer gives the reasons for assuming that the glomerular membrane is less permeable at the same time as a small number of its pores is larger than normally, thus allowing the passage of proteins and red blood cor-

puseles. It is also possible that an increased tonus in the afferent arterioles as well as an increased tissue pressure contribute towards decreased filtration. Moreover, the figures show that the function both of the tubules and of the glomeruli is decreased and that both units are presumably damaged.

In *essential hypertension* the results of the functional tests are quite the opposite. The writer concludes — as do earlier workers — that there must be an increased tonus in the efferent arterioles and also possibly a change in the excretory power of the tubular cells. At later stages there is reason to assume that the permeability of the glomerular membrane also undergoes changes.

In *chronic nephritis* there is some indication that changes of both the forementioned types are present, the former being predominant at times and the latter at others.

10. The writer demonstrates that there is a strong *correlation* in his material *between the inulin and the creatinine clearances*. There is also a correlation between the inulin and urea clearances and between the former and the maximal concentration capacity, although this is less pronounced. There is no demonstrable correlation in the writer's material between diodrast clearance and the maximal concentration capacity.

11. A calculation of the *correlation between inulin clearance and the level of non protein nitrogen or urea nitrogen* reveals that in this material (61 and 219 cases respectively) the non protein nitrogen begins to rise above the upper limit only when the clearance falls to approximately 30-40 ml/min, whereas the urea nitrogen rises at values around 50 ml/min.

12. On the basis of the tests performed, the writer concludes that even in marked renal injury inulin clearance is a satisfactory measure of the filtration rate. It is thus not necessary to postulate an increased permeability of the tubular cells in still functioning nephrons in order to explain why the clearance values for inulin, creatinine and urea tend to converge under these conditions.



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## RENAL FUNCTION IN DIABETIC NEPHROPATHY

BY

*OLLE HOGEMAN*

ACCOMPANIES VOL. CXXXII (132)

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FROM THE MEDICAL CLINIC OF THE UNIVERSITY OF UPSALA  
(HEAD: PROFESSOR ERIK ASK-UPMARK, M. D.)

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RENAL FUNCTION  
IN  
DIABETIC NEPHROPATHY

BY

OLLE HOGEMAN



UPPSALA 1948  
APPELBERGS BOKTRYCKERI AB





## RENAL FUNCTION IN DIABETIC NEPHROPATHY

Diabetes mellitus is one of the diseases in which renal complications often occur, and it can be questioned whether they are more common in any other chronic disease. Various causal factors may be responsible, but the three following seem to be the principal ones.

1. Factors connected with diabetic disturbances in metabolism, for example, renal changes accompanying acidosis or coma and glycogenic nephrosis.

2. Changes directly dependent on the increased susceptibility of diabetics to infection, among which infections of the urinary tract are the most important. Such post-infectious diseases, e.g. acute glomerulonephritis, may perhaps be included in this category.

3. Changes that are generally considered to accompany arteriosclerosis but whose aetiology must still be considered as obscure. Diabetic nephrosclerosis and intercapillary glomerular sclerosis belong to this group.

The relation of diabetes to arteriosclerosis is obscure. Although no exact figures exist for the frequency of arteriosclerosis in the normal population, we nevertheless have the impression that this disease is frequent in connexion with diabetes mellitus. One of the principal reasons may be that many diabetics belong to the higher age groups, when arteriosclerosis is common and, according to a widespread opinion, the prognosis is then relatively good. It is also possible that arterio-

sclerosis *per se* causes certain disturbances which in turn give rise to diabetes. It is, however, also possible that the metabolic changes characteristic of diabetes can favour the onset of arteriosclerosis which then becomes manifest earlier and more frequently in such patients than in healthy individuals. Without entering more deeply into the problem, the foregoing statements are made only to emphasize that the problem is as yet far from solved.

Renal function in diabetics who show no signs of renal disease—termed normal diabetics in the following—will now be discussed. The writer was able to find only one investigation in the literature in which clearance determinations were performed on such patients, i.e. that of Cambier (1934). He investigated 20 normal diabetics between the ages of 18 and 70 years and used 10 normal individuals (aged 19–47 years) as comparative material. He found high filtration figures, determined by creatinine clearance, in the majority of the diabetics, and the clearance figures for the whole group were higher than for the normal material. Cambier assumed that the abnormal figures were caused in some way by the hypophysis. He did not, however, give any details of the supposed mechanism, but his conclusions appear to have been drawn by analogy, since he also found a sub-normal urea clearance in diabetics as well as in five cases of acromegaly (three without glycosuria), i.e. raised creatinine clearance and sub-normal urea clearance. When he injected pituitrin into three patients (one diabetic, one with acromegaly and one a normal individual) the conditions were reversed, i.e. the creatinine clearance sank and the urea clearance rose. Cambier's general conclusions cannot be accepted without further investigation since his material was so small and the differences found must therefore be somewhat uncertain.

The present writer investigated twelve diabetics without nephropathy (five men and seven women). Data concerning their age are found in table I and the duration of the diabetes in table II. To evaluate the results normal values must be used. In another investigation (Hogeman 1948) such determinations were made on 56 individuals (between the ages of

Table I

Distribution according to age in cases of diabetes with and without nephropathy. Figures in brackets indicate deceased cases (they are included in the figures without brackets).

Age in years	Diabetes without nephropathy		Diabetes with nephropathy	
	Men	Women	Men	Women
$\leq 20$	1	0	0	0
21-30	2	1	2(1)	2(1)
31-40	0	2	1	1
41-50	1	1	0	1(1)
51-60	0	1	0	2
61-70	1	1	3	0
$\geq 71$	0	1	0	0
Total no.	5	7	6(1)	6(2)

Table II

Distribution according to the duration of the disease in cases of diabetes with and without nephropathy. Deceased cases in brackets as in Table I.

Duration in years	Diabetes without nephropathy		Diabetes with nephropathy	
	Men	Women	Men	Women
$\leq 1$	2	2	0	0
1-3	1	1	0	0
3-6	1	2	0	0
6-9	0	0	0	1
9-12	1	0	2	0
12-15	0	2	2(1)	1(1)
15-18	0	0	0	2
$> 18$	0	0	2	2(1)
Total no.	5	7	6(1)	6(2)

13-17 years) and the following values were obtained for the sexes together:

	$M \pm r(M)$	$\sigma$
Inulin clearance:	$122.2 \pm 1.8$	13.2
Diodrast clearance:	$444.2 \pm 5.7$	41.6
Filtration fraction:	$0.2753 \pm 0.0041$	0.0298

If the means are calculated for diabetics without nephropathy the following results are obtained:

	$M \pm r(M)^1$	$\sigma$
Inulin clearance:	$117.0 \pm 11.7$	15.4
Diodrast clearance:	$394.8 \pm 35.4$	68.3
Filtration fraction:	$0.300 \pm 0.021$	0.043

These means do not differ very much from the normal values. The differences found can be due to random variation.

Cambier's hypothesis was in no way confirmed by the figures obtained and — as far as one may venture to draw conclusions from the present material — diabetes mellitus does not *per se* necessarily cause any demonstrable disturbance in renal function.

As mentioned in the beginning of this paper, there are several reasons why diabetes is often associated with renal complications. Only the conditions under point 3, i.e. intercapillary glomerular sclerosis and so-called diabetic nephrosclerosis, will however be discussed in the following. The present writer prefers to use the term »diabetes with nephropathy» for the latter disease, for reasons which are evident from the following.

Glomerular sclerosis, which was first described by Kimmelstiel and Wilson (1936), has aroused great interest in recent years, despite its rare occurrence, since the disease is characterized by a definite clinical syndrome (often moderate diabetes in patients over 40 years of age, heavy proteinuria.

<sup>1</sup> The standard error is calculated here and on page 10 from the standard deviation of the total number of diabetics with and without nephropathy and not from the standard deviation given here. The total standard deviation is thus for inulin clearance 40.5, for diodrast clearance 122.5 and for the filtration fraction 0.074.

pronounced oedema of the nephrotic type, hypertension and retinopathy) with a poor prognosis. Kimmelstiel and Wilson described eight cases. Since then several cases have been reported. It can be questioned whether there is actually a particular syndrome or whether it is rather a selection of cases with nephropathy and a poor prognosis. It is evident that there are many degrees of this disease, from insignificant lesions to widespread degeneration, and that its course can vary from a practically stable condition to a very malignant progression. In the future it will possibly be easier to make the diagnosis if the hypothesis that certain vascular lesions, proliferative retinitis and rubeosis iridis (v. Bah r 1947) occur simultaneously with this intercapillary diabetic glomerular sclerosis proves to be correct.

The usual occurrence of »nephrosclerosis» in diabetics was investigated by Joslin (1917) and later by von Noorden (1927) and by Fahr (1937), amongst others. According to Bechgaard (1946) this type of »nephrosclerosis» gives rise to renal and ocular symptoms similar to those in malignant nephrosclerosis but differs from the latter in the following respects. The course is more benign, the distribution according to age and sex is entirely different and the blood pressure is, on the average, only slightly elevated. His opinion was based on 22 cases of diabetes with renal disease. Eleven of them were alive at the time of the investigation and eleven had died 0-9 years after diabetes had been diagnosed. Of these eleven, one had died of cancer of the rectum, three of cerebral haemorrhage or coronary thrombosis and the remainder of renal disease. All the patients had a moderate elevation of the blood pressure, proteinuria and retinal changes. Urea clearance determinations were made in 11 of the 22 cases. Four had values over 60 per cent and in the remainder they varied between 9 and 57 per cent. Bechgaard concluded that nephrosclerosis and diabetic retinitis »apparently accompany each other to a strikingly high degree». He did not, however, go as far as other writers who consider that diabetic retinitis cannot occur without simultaneous hypertension and vascular changes (Volhard 1921, Wagner and Wilder 1921,

von Noorden 1927, Bessière 1932). Hansen (1946) found in his material that of twelve patients with constant proteinuria nine had considerable hypertension, retinitis, a rise in blood urea or angina pectoris. No renal functional tests were, however, made on these patients. Of the 19 patients with retinitis who died, the cause of death was renal disease in seven cases and cerebro-vascular or cardiac disease in nine. Hansen drew the conclusion from this investigation that constant proteinuria is associated with marked arteriosclerosis.

Three principal questions arise in a discussion of diabetes and retinopathy: 1) Is there any connexion between these conditions and proteinuria? 2) Or between retinitis and impaired renal function? 3) Is hypertension the aetiological factor in the occurrence of retinitis?

A number of investigations have been made to determine how often diabetic retinitis is associated with proteinuria. Onfray (1922) found proteinuria in 41.5 per cent of his cases of retinitis, Dirion (1933) in 57 per cent, Cambridge (1930) in 45.5 per cent, Bessière (1932) in 89 per cent, Gresser (1933) in 79.5 per cent and Hannum (1938) in 47 per cent. As a comparison with these figures, it should be mentioned that the occurrence of proteinuria in diabetics has been assessed at a very high figure by a number of writers. Hattelhol (1924), for example, gives approximately 50 per cent, O'Donoghue (1931) 77 per cent, whereas others, on the contrary give much lower figures, i.e. Isaac and von Noorden (1927) 21.5 per cent and Hannum (1938) 16 per cent.

It is apparent from the investigations reported here that diabetic retinitis can occur without simultaneous proteinuria and that the question of the frequency of proteinuria in such cases does not appear to have been established. In any case, different writers make very divergent statements.

Another important question is obviously whether diabetic retinitis *per se* is closely connected with impaired renal function. Opinions appear to differ. Russo (1925) found, for example, decreased function in 9 out of 24 patients, but it was not possible for the present writer to ascertain which functional

tests were used. Onfray (1922) found an increase of Amhard's constant (= impaired renal function) in 12 out of 17 patients and Wagener and Wilder (1921) found lowered secretion with the phenolsulphonphthalein test according to Rowntree and Geraghty in 22 out of 28 patients. Gresser (1933), using the same method, found a decrease in 31 out of 33 patients. Hanum (1938) and Hanum and Brøchner-Mortensen (1938) investigated 35 cases of diabetic retinitis by means of urea clearance determinations as well as creatinine and uric acid clearance in some cases. With the relatively wide range of variation given by them, they only found decrease of function in one of their 35 cases. They therefore concluded that »retinal changes can develop in diabetics even if their kidneys function quite normally» and were of the opinion that the materials of earlier workers were possibly selected and thus not representative. Nevertheless, on perusal of the tables in the forementioned writers' retinitis material (183 cases) it is seen that there were altogether 14 cases that also had constant proteinuria and elevation of the blood pressure (190/100 mm Hg or more). Renal functional tests had only been made in two of these 14 cases (11 women and 3 men, aged between 49 and 74 years). The urea clearance was 146 per cent in one (a 59-year-old woman who had suffered from diabetes for 7 years) and 55 per cent in the other (a 67-year-old woman with diabetes of 5 years' standing) and a simultaneous creatinine clearance of 75 ml per minute. There were a further 15 of these 183 cases that had proteinuria but no elevation of the blood pressure.

The last question of importance in this connexion is whether hypertension can *per se* be of decisive importance for the development of diabetic retinitis; in other words, whether Volhard's hypothesis that »without hypertension there is no retinitis in diabetics» is true. Hanum (1938) after a critical summary of the literature and of his own material, came to the following conclusions:

1. The occurrence of hypertension is percentually more frequent in diabetics with retinitis than in diabetics without retinal changes.



2. Moreover, the tendency towards relatively more marked increase of the blood pressure with advancing age seems to be greater in the former group of diabetics.

3. This circumstance does not permit the conclusion that hypertension should play a part in the aetiology of retinitis.

It is evident from this short survey of the literature that the aetiology of diabetic retinitis is obscure. Its elucidation is rendered more difficult by the fact that the disease exhibits different types and different courses in various patients. In order to solve this problem, a large material is necessary with a thorough investigation of the duration of the diabetes and the time of its onset, the degree of the symptoms (glycosuria, blood sugar levels, acidosis, etc.), the changes in the blood pressure and the fundi, renal function and — not least important — the treatment of the disease. The present writer does not intend to report such an investigation in the present study, since it would have to be on a very long-term basis. The aim of the present investigation was to investigate the renal function in a group of diabetics, who fulfill certain determined criteria for particular changes, in order to ascertain the relation of diabetes to such changes and possibly to obtain guidance for the prognosis.

The following criteria were set up:

1. Constant proteinuria
2. Elevation of the blood pressure
3. Retinitis.

It was not possible to take into consideration the various types of retinitis exhibited by the patients, but the changes were nevertheless considerable and bilateral in all the cases, consisting of vascular changes, haemorrhages and often exudate.

The material was small, consisting of twelve cases (6 men and 6 women). The age-distribution is seen in table I and the duration of the diabetes in table II. The following mean figures and standard errors were obtained for this group:

	$M \pm \epsilon(M)$	$\sigma$
Inulin clearance:	$59.2 \pm 11.7$	37.1
Diodrast clearance:	$270.9 \pm 35.4$	135.4
Filtration fraction:	$0.206 \pm 0.021$	0.070

It can be seen that there is a statistically significant difference for all three values as compared with normal individuals and thus lower inulin and diodrast clearances. The filtration fraction is also lower than normal. If a comparison is made with the small number of cases of diabetes without nephropathy, significant differences are also found, except for the diodrast clearance, in which the difference is nevertheless probable. There is little reason to doubt that, with a larger material, this difference would also be significant.

In order to throw further light on the problem, a calculation was made of the number of cases falling outside the normal range of variation. This was made on the basis of the values previously given and using different strict limits, i.e.  $2\sigma$ ,  $2\frac{1}{2}\sigma$  and  $3\sigma$  from the mean value. The results are seen in table III. It is seen that if the limit is very strict, i.e. at  $2\sigma$ .

Table III

Number of individuals suffering from diabetes mellitus with and without nephropathy falling outside the normal limits calculated according to  $2\sigma$ ,  $2\frac{1}{2}\sigma$  and  $3\sigma$  respectively.

Determination	$M \pm 2\sigma$				$M \pm 2\frac{1}{2}\sigma$				$M \pm 3\sigma$			
	Below	Between	Above	Total no.	Below	Between	Above	Total no.	Below	Between	Above	Total no.
<i>Diabetes without nephropathy</i>												
Inulin clearance . . . . .	1	11	—	12	1	11	—	12	—	12	—	12
Diodrast clearance . . . .	4	8	—	12	2	10	—	12	1	11	—	12
Filtration fraction . . . .	—	9	3	12	—	11	1	12	—	11	1	12
Creatinine clearance . . . .	1	2	—	3	—	3	—	3	—	3	—	3
Urea clearance . . . . .	—	3	—	3	—	3	—	3	—	3	—	3
Urea nitrogen, mg % . . . .	—	5	1	6	—	5	1	6	—	6	—	6
<i>Diabetes with nephropathy</i>												
Inulin clearance . . . . .	11	1	—	12	9	3	—	12	8	4	—	12
Diodrast clearance . . . .	8	4	—	12	8	4	—	12	8	4	—	12
Filtration fraction . . . .	8	3	1	12	8	4	—	12	6	6	—	12
Creatinine clearance . . . .	5	3	—	8	5	3	—	8	5	3	—	8
Urea clearance . . . . .	—	—	—	—	—	—	—	—	—	—	—	—
Urea nitrogen, mg % . . . .	—	1	—	1	—	1	—	1	—	1	—	1

individual values fall below and above it in the case of the group with diabetes without nephropathy, although the majority fall within the limits. This displacement towards abnormal values is less pronounced if the borderline is drawn at  $3\sigma$ . Then only one value, i.e. that for diodrast clearance, falls below the normal range of variation and one, the filtration fraction, lies above it. This can depend on the fact that the normal values are taken for young and undelayed individuals between 13-17 years of age, but that several older persons are included in this material.

When we are dealing with the group of diabetics with nephropathy, an entirely different picture is found. As the table shows, the majority of values fall below the normal limit, even if this is drawn at  $3\sigma$ . On the whole it can be stated that between three-fourths and half of the values fall below this limit. It can further be pointed out that whereas for uncomplicated diabetes single values above the normal are found in respect of the filtration fraction, this is not the case for diabetics with nephropathy. It should be recalled that this is, on the contrary, a common phenomenon in nephrosclerosis and hypertension.

### *Discussion*

It is already an established fact that a definite correlation exists between diabetes accompanied by retinitis, and proteinuria, hypertension or impaired renal function, the last-mentioned assessed by means of the phenolsulphonphthalein test or urea clearance. Only one previous investigation (Bechgaard 1946) has been made on renal function in diabetics suffering from retinitis with accompanying hypertension and constant proteinuria. The present writer therefore considered it permissible to report the findings in the present material, despite its small size, especially as such functional investigations on diabetics have not been published earlier.

The investigation showed that impaired renal function was present in the diabetics with constant proteinuria, elevated blood pressure and retinopathy if a calculation of the figures for these functions was made for the group as a whole. In a

further analysis it was found that there was a very much larger variation in the figures in this material as compared with normal or »normal diabetic» material. This is conditioned by the fact that there were considerable variations between the individuals belonging to this group and it can therefore be assumed that some of the individual patients showed normal figures. There was no such case among the women, but among the men there was one — the youngest of the patients — who had entirely normal figures at the time of the investigation. Practically identical values were obtained at an examination three months later, thus proving that it could not have been a question of a technical error. Nevertheless, this 20-year-old man died of true uraemia eighteen months later. Of the remaining men, two had a slight decrease in renal function, whereas the rest had moderately to exceedingly pathological values.

The majority of patients in this group showed a considerable deterioration in renal function, but the correlation between the degree of deterioration and retinopathy is not absolutely uniform even in this selected material, despite the presence of clinical symptoms indicative of renal injury. Moreover, the fact that retinopathy may occur without any perceptible change in renal function is illustrated by the following two cases that were not included in the material since, although proteinuria was present when they were admitted to hospital, it disappeared during their stay there. They were not therefore considered to fulfill the conditions earlier set up for this group.

1. *A man, aged 40 years with diabetes of 10 years' standing and retinitis diagnosed 3 years previously. Blood pressure: 155/110 mm Hg. Proteinuria: 0.3 promille on admission, disappeared after about one week. Sediment: a few red and white blood corpuscles. Inulin clearance: 130 ml/min. Diodrast clearance: 140 ml/min. Creatinine clearance: 139 ml/min. Effective renal blood flow: 687 ml/min.*

2. *A woman, aged 48 years with diabetes of 8 years' standing. Retinitis diagnosed at present investigation. Blood pressure: 130/90 mm Hg. Proteinuria: 0.3 promille on admission, disappeared after about one week. Sediment: N. A. D. Inulin clearance: 117 ml/min. Diodrast clearance: 378 ml/min. Effective renal blood flow: 630 ml/min.*

The present investigation has afforded more definite proof that diabetic retinitis is not necessarily associated with an impairment in renal function, at any rate not to a degree possible to reveal with the methods used here. Earlier investigations have shown that proteinuria need not constantly be present and that retinitis can occur without simultaneous hypertension. Thus Volhard's dogmatic opinion described in the foregoing is not supported. There is one further possibility, i.e. that retinitis, proteinuria and hypertension and also impairment of renal function are all co-ordinated symptoms of one and the same organic lesion caused by the metabolic changes of diabetes mellitus. In this case sometimes one symptom and sometimes another can occur initially but in advanced cases the entire syndrome is present. This theory is further supported by the fact that all the patients in this group in the present investigation had suffered from diabetes for many years, the majority for between 12 and 20 years. It is, nevertheless, impossible as yet to establish the nature of this aetiological factor or whether there are several contributory factors.

In advanced cases these patients present the clinical picture of malignant hypertension, with the possible exception that the degree of elevation of the blood pressure need not necessarily be high (Bechgaard 1946). This author nevertheless pointed out an intrinsic difference between malignant hypertension and this diabetic nephropathy, i.e. the prognosis; which in the latter case is more comparable with that for benign hypertension. The number of deaths in the present writer's material was 25 per cent. As a comparison it can be mentioned that the number of deaths in the group with malignant hypertension (retinal changes III-IV) was 72 per cent during approximately the same time of observation (Hogeman 1948).

Although the material is very small the difference was probable ( $48 \pm 18\%$ ). There are consequently quite good prospects that the results will be confirmed by further investigation.

The present writer wishes to point out yet another difference, namely the age. In the present material two patients died at the age of 24, thus at an age which is scarcely found in a material consisting of cases of malignant hypertension. That a

relatively rapid deterioration can occur in cases in this group is demonstrated by the fact that in one of the patients, as mentioned earlier, renal function became so impaired that eighteen months after having normal figures in this respect he died of uraemia.

Finally, the filtration fraction must be considered. As is seen on page 10 the mean figure is  $0.206 \pm 0.021$  for this group, with a standard deviation of 0.070. This is low in comparison with the figure for the normal material. If this is compared with the figure for hypertensives, or perhaps even better with that for the group with retinal changes III-IV (malignant hypertension), it is very striking that it is so low for this diabetic group whereas it is considerably raised in the group of hypertensives and very particularly in those with malignant hypertension. This is all the more strange since, as pointed out in the foregoing, diabetic nephropathy and malignant hypertension show a clinically very similar picture. What is the reason for this fact?

It can scarcely be explained by mere chance since, although the material was small, only two of the 12 cases showed figures over the normal; the majority were under 0.20. Of the four patients who showed figures over 0.20, two died of renal insufficiency and the other two were 64 and 65 years old respectively, thus at an age when the »age factor» contributes to raising the filtration fraction. If, therefore, we first consider the purely clinical similarity between the patients suffering from diabetic nephropathy and those with malignant hypertension and then the low filtration fraction which is more reminiscent of conditions in acute nephritis, we obtain a possible indication of why the prognosis seems to be so much better for the patients with diabetic nephropathy than for those with malignant hypertension. There is a strong impression that the mechanism causing the decrease in renal function is entirely different in the respective groups and that — if such an expression can be used — the »malignant phase» of diabetic nephropathy consists of Kimmelstiel and Wilson's intercapillary glomerular sclerosis.

### Summary

Renal function is studied in 12 cases of uncomplicated diabetes and in 12 individuals suffering from diabetic nephropathy in the form of proteinuria, positive urinary sediment and elevation of the blood pressure as well as retinal changes.

Determinations are made of the inulin and diodrast clearances and, also, in a few instances, of the creatinine and urea clearances and the non protein nitrogen level in the plasma.

The writer comes to the following conclusions:

1. Renal function in *uncomplicated diabetes* does not deviate from the normal.

2. In form of *diabetic nephropathy* studied here, there is on the other hand, *usually* a change in renal function towards pathologically decreased values.

3. A comparison is made between these latter cases and the results of similar functional tests in individuals suffering from malignant hypertension, which shows the same clinical picture.

Despite this, cases of diabetic nephropathy show a *better prognosis* than those with malignant hypertension. The filtration fraction (the relation between the inulin and diodrast clearances) is *high* for the latter, whereas in the majority of those with diabetic nephropathy it is *low*. This is similar to the findings in acute nephritis if the filtration is decreased.

4. This fact, combined with the more favourable prognosis in such cases, is considered by the writer to be associated with the *different localization of the injury* in the two diseases. The hypothesis is put forward that the »malignant phase» of diabetic nephropathy consists of Kimmelstiel and Wilson's glomerular sclerosis.

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This paper has a close connexion to Supplementum CCXVI a, published in this Journal.







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## MYOCARDIAL INFARCTION AND MURAL THROMBOSIS IN THE ATRIA OF THE HEART

BY

*NILS SÖDERSTRÖM*

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MURAL THROMBOSIS  
IN THE ATRIA OF THE HEART

BY

*NILS SÖDERSTRÖM*

UPPSALA 1915  
ALMQVIST & WIKSELLS BOKTRYCKERI AB



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## Preface.

This investigation has been carried out during the author's service in the Medical Clinic at the University Hospital, Uppsala. It is my pleasant duty to thank my teacher Professor GUSTAF BERGMARK, the former chief of the Clinic for his interest and support during the first years of this investigation. My gratitude is in a like measure due to the present chief of the Medical Clinic, Professor ERIK ASK-UPMARK for his help and encouragement.

A considerable part of the work has been performed at the Institute of Pathology at the University of Uppsala. I here wish to thank Professors ROBIN FÄHRÆUS and NILS GELLERSTEDT for their interest in my work and for their valuable advice.

Autopsy specimens from other pathological departments have been placed to my disposal by Professor H. BERGSTRAND, Drs. O. FORSELIUS, G. VEJLENS, N. RINGERTZ, and J. MELLGREN.

I had access to hospital records regarding a great number of patients by the courtesy of Professors M. ODIN and G. NYLIN, Drs. B. STRANDELL, B. EWERT, and S. ECKERSTRÖM.

Mr. E. LANDER, actuary at the State Institute for Human Genetics and Race Biology at Uppsala, has given me valuable assistance in the statistical treatment of the material.

The revision of the English text was competently carried out by Dr. ANN SYNGE, Aberdeen, and by Mr. K. M. LINDSKOG, Uppsala.

All these persons and others, who directly or indirectly have aided me in my efforts, deserve my deepest gratitude.

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## Introduction.

The original purpose of this investigation was to study the clinical signs of infarcts in the atria of the heart. The author had seen some cases with a suggestive clinical picture, but autopsy confirmation was not obtained. The author then decided to go about it in the opposite way, collecting a material of proved atrial infarcts from autopsies and looking for corresponding clinical signs in the hospital records. It may be mentioned here that the investigation with the same scope of CUSHING, FEIL, STANTON and WARTMAN (1942) was not known to the author (because of the war and post war conditions) until 1946, when the present study was rather advanced.

The author expected atrial infarcts to be rare (which was indicated by the scanty literature on the subject) but otherwise commensurable with the large zones of complete necrosis in the ventricular walls, which are generally imagined by physicians when speaking about myocardial infarcts. The diagnosis of myocardial infarcts at autopsy is known not to be difficult, and the author hoped that the sampling of a sufficient number of atrial infarcts in an autopsy material would be a comparatively easy procedure.

Experience did not justify this hope. Atrial infarcts proved to be difficult to diagnose and practically impossible to recognize with the naked eye. Suspicious based on the clinical signs or on the gross appearance at autopsy proved as a rule to be ill-founded on histological examination. Not until the author realized the value of mural thrombosis as an index of underlying atrial infarction did the number of cases registered begin to rise. The sampling was then based upon histological examination of cases with atrial mural thrombosis. Only a fraction of the cases of thrombosis yielded atrial infarcts, but the observations on this material made it possible for the author to survey the hitherto neglected question of the etiology and development of atrial mural thrombosis.

Not only the gross recognition but also the histological diagnosis of atrial infarcts proved to be difficult. This led the author to devote some study to the histological picture of "coronary" myocardial lesions, especially the less advanced ones, which also represent a neglected topic in previous literature.

The histological findings not only elucidated questions regarding atrial infarcts, but contributed in some ways to the knowledge of "coronary"<sup>1</sup> heart lesions in general. Consequently the morphology of "coronary" lesions in the atria has become one of the main themes of the present study.

Clinical signs, referable to atrial infarcts, proved to be rather scanty, sometimes diffuse and as a rule of little practical interest. The clinical observations have consequently become a less important part of the study than was originally expected.

It is evident from this survey that the present study may seem composed of rather heterogenous elements. They are however kept together by one plan which has remained the main thread through the present investigation: to study the effect of deficient coronary blood supply to the atrial myocardium.

## CHAPTER I.

### Some general considerations concerning the site of myocardial infarcts.

It seems to be generally accepted in the literature, that myocardial infarcts are found almost exclusively in the walls of the *left ventricle*.

There has not been much discussion about this fact which is nevertheless remarkable. According to figures to be found in P. D. WHITE's manual the weight of the atrial and the right ventricular walls together nearly equals that of the walls of the left ventricle. Thus,

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<sup>1</sup> The word "coronary" will in this study often be used in the sense: "caused by a deficient supply of blood through the coronary arteries". The author is quite aware of the fact that this use of the word "coronary" is not correct. In order to avoid long circumlocutions, the author will however go on using this word (with quotation marks) in this sense.

myocardial structures, representing, in the normal heart, about half the total myocardial mass would appear to be practically immune to a type of lesion, which is of dominant importance in the other half.

A number of reasons can be advanced to explain this discrepancy.

In the actual cases there is unquestionably often a left ventricular hypertrophy. On purely statistical grounds this fact would account for a preponderance of the left ventricle as site for infarcts, if they were assumed to be scattered at random over the total myocardial mass.

Some authors assume (e. g. HOLZMANN and others), that *the sites of predilection for coronary narrowing or thrombosis* may account for the uneven distribution of infarcts. To the present author this explanation does not seem very satisfactory. It is true, that according to most statistical studies, the ramus interventricularis of the left coronary artery is somewhat more often occluded than the other coronary arteries. This artery is known to be *mainly* a nutrition artery of the left ventricular wall. But it is unquestionable that in the main stems of the coronary tree there is no place where an occlusion can affect exclusively the nutrition of the left ventricle without also interfering gravely with the circulation in the walls of some other chamber of the heart.

To the best of the author's knowledge it has never been suggested that *intercoronary* arterial anastomoses are less well developed in the left ventricle than in the rest of the heart. The anastomoses existing in the normal heart have been the object of numerous investigations and animated discussion (see SPALTENHOLZ) but are probably of little immediate functional importance. Wide and certainly important collaterals observed by SCHLESINGER and BLUMGART and others are regarded by these authors as having probably developed gradually during the slow progress of a coronary sclerosis, to meet the local demands for collateral blood supply in all parts of the heart.

As regards the possibilities of nutrition by means of extracoronary vessels or directly from the cardiac lumen the left ventricular myocardium is probably at a disadvantage as compared with the walls of the atria. This matter will be discussed more in detail in subsequent chapters.

The explanation which seems most attractive to the present author

is based on the current *clinical* conception of *coronary insufficiency*. The classical definition of this conception is a disproportion between the actual demand and the supply of oxygen to the myocardium. The myocardium seems to respond immediately to all demands for increased work without having any guarantee of the availability of the increased oxygen supply which will become necessary at least in the long run. One of the clinical signs of this discrepancy is anginal pain; it has been shown by BUECHNER and his coworkers that actual myocardial necrosis may be the anatomical consequence. We have been accustomed to resort to explanations of this kind in those not uncommon cases in which there is myocardial infarction but no coronary thrombosis or even actual narrowing of the coronary arteries.

Sudden stress would be expected to be the immediate cause of such infarcts and the part of the heart most often exposed to sudden stress is indubitably the left ventricle. In a case of coronary occlusion the area of defective nutrition would not theoretically be expected to respect the limits between the heart chambers. During rest collateral circulation may possibly be sufficient to prevent necrosis throughout this area. Every activity, however, immediately places a burden on the *left* ventricle, and the necrosis may thus become manifest in its walls while remaining latent in the other working units of the heart, which may be assumed to be more sheltered from the immediate effect of such a sudden overburdening.

These reasons put together may seem to be sufficient to explain the extra proneness of the left ventricle to myocardial infarction. However it must not be forgotten that left ventricular infarcts are prone to give rise to impressive clinical signs and are often fatal in the acute stage. They are consequently common findings in autopsies, and particularly easy to recognize in the broad mass of left ventricular myocardium. There is reason to believe that infarcts in other sites are likely to escape attention, clinically as well as post mortem. Occasional microscopical examinations of the right ventricular myocardium in infarct cases have made the author think that this assumption may be true for the right ventricle. The possibility that such oversights have been made in respect of the atria will form the main subject for study in the following chapters.

## CHAPTER II.

## The literature concerning atrial infarcts.

## a. Observations in cases verified by autopsy and in experimental studies of atrial infarction.

Owing to the supposed rarity of the condition, most reports concerning atrial infarcts in literature are descriptions of single cases observed in autopsies.

The first case was described by CLERC & LEVY in 1925. The case was a man, 60 years old, with mitral stenosis and auricular fibrillation, who actually presented the clinical picture of an acute congestive failure. It is uncertain if the fibrillation ("fibrilloflutter") was acute or chronic. At autopsy a hemorrhagic infarct was observed in the right auricle ("*région supéro-extérieure*").

Some years later LISA & RING (1930) reported a case in which the immediate cause of death was a spontaneous rupture of the left auricle. At the site of the rupture the myocardium showed advanced degeneration (fibrous scars and foamy muscle fibres). There was an extensive coronary sclerosis, thrombosis of the ramus interventricularis of the left coronary artery and multiple, recent and old ventricular infarcts. The histological picture of the ventricular lesion was similar to that found in the left auricle. An ECG taken 2 weeks before death showed a regular sinus rhythm and nothing abnormal in the atrial part of the ECG.

In a case published by CLOWE, KELLERT & GORHAM (1933) there was a ruptured infarct of the right atrium. No conspicuous changes were noted in the main coronary arteries, and no recent ventricular infarct was found. Microscopical sections showed a distinct intimal thickening in the minor arteries (both in the atrial and the ventricular walls) "with considerable disorganization due to edema in the subpericardial tissue and muscle and many polymorphonuclear cells". The clinical picture was that of an acute myocardial infarct (persistent precordial pain of sudden onset and acute cardiac failure). The patient died 4 days after his admission to the hospital; 3 ECG's were obtained during this time. The authors did not find anything peculiar in the atrial part of the ECG, the present writer notes that the P-Q-time, which was in the first ECG (4/4 1930) 0.12 sec. had

in the last ECG (7/4 1930) shortened to 0.10 sec. (see p. 88 in the present study).

Another case with rupture of the right auricle was described by LAIGNEL-LAVASTINE, LIBER & BIDOU (1934). In this case there was a recent occlusive thrombosis of the right coronary, infarction of the posterior wall of the left ventricle and a ruptured infarct of the right auricle. These authors noted that in the auricle the myocardial damage was more pronounced under the epicardium than near the endocardium. There was no ECG in this case and the rhythm was referred to only as "tachycardia".

Nothing had been learnt by these earlier reports about the possibility of the existence of a specific ECG picture of atrial infarcts. This question was taken up by LAMBERT (1937) who studied the ECG alterations in 16 rabbits after occlusion of atrial arteries by means of artery clips. According to LAMBERT there are, in rabbits, only 2 atrial arteries of importance, originating from the left and right coronary, respectively, soon after they leave the aorta. The observations were only made during the course of the operation and the occlusion of the arteries was maintained for varying periods of time (seemingly as a rule one or two hours). ECG changes were registered after some minutes. The most important ones were:

- a. In some cases a depression of the P-Ta level (sometimes conspicuous) in the limb leads II—III. It apparently made no important difference whether the clips were placed on the right or the left artery only or on both simultaneously.
- b. In other cases there were changes in the contour and direction of the P-waves. LAMBERT noted specially negative or diphasic P-waves in leads II and III. In cases in which the P-Q-intervals were shortened, he regarded the negative P-waves as consequences of a nodal rhythm, if the P-Q-intervals were unchanged he assumed the negative deflections at the site of the P-waves to be analogous with the "coronary T-waves" of myocardial infarcts. The latter explanation is somewhat difficult to understand and has not been accepted by other authors. (LANGENDORF, SEILLER and WEISSEL.)
- c. In some cases an irregular atrial tachycardia denoted by the author by the term *arrhythmie anarchique*.

LAMBERT also saw depression of the P-Ta intervals in leads II—III after crushing of the right auricular wall.

Finally, LAMBERT reproduces ECG's from 8 patients with coronary heart disease in varying stages, exhibiting depressed P-Ta-intervals or negative P-waves somewhat similar to those found in the rabbit experiments. In none of these cases did LAMBERT have the opportunity of post mortem verification of the suspected atrial lesions.

Some similar cases were later published by LAMBERT & REIGNER (1938) and by REIGNER (1939), without adding new facts of interest for the solution of the problem. The present author has, however, only seen abstracts of these papers.

In 1938 FEIL, HARDESTY & CUSHING found 2 cases of right atrial infarct in a series of 34 cases of myocardial infarct. In both cases ECG's were available. One case had auricular fibrillation, the other had regular sinus rhythm and no conspicuous changes in the atrial ECG. In both cases there were multiple coronary arterial thromboses and posterior wall infarcts of the ventricles.

In the same year ABRAMSON, FENICHEL & SHOOKHOFF studied the ECG's of 6 cats and 5 dogs after canterizing of the atrial wall. They found that cauterizing the left atrium was followed fairly regularly by a depression of the P-Ta-level in leads II—III and an elevation of the same level in lead I. Cauterizing the right atrial wall was followed by a depression of the P-Ta in *all* the limb leads. Changes in rhythm or in the configuration of the P-waves seem not to have been conspicuous in this series.

In a similar investigation SANDERS (1939) caused destruction of the atrial myocardium in dogs by intramural injection of 95 per cent alcohol. Injections into the walls of the left atrium resulted as a rule in elevation of the P-Ta in lead I and depression in leads II—III. Injections into the walls of the right atrium were also followed by depression of P-Ta in II and III, but no changes were observed in lead I. The P-Ta levels in the esophageal lead often became positive, especially in rightsided lesions (depressed in the coupling used by this author). Furthermore he noted numerous changes in the contour of the P-waves: decrease of amplitude, broadening, slurring, notching etc., occurring with varying frequency. The occurrence of diphasic or triphasic P-waves was denoted by this author as the development of atrial Q-waves or M- and W-shaped P-waves in order to mark an analogy with the well known corresponding conceptions in the ventricular part of the ECG in cases of ventricular infarction.



LANGENDORF (1939) was the first to report a case of atrial infarction, in which there had been during life an ECG suggestive of this lesion. His case was a woman of 63 years who died showing the clinical picture of an advancing congestive failure. At autopsy a severe coronary sclerosis was found with total occlusion of the left and partial occlusion of the right coronary artery. The myocardium showed numerous scars in the left ventricle and recent infarcts in the right ventricle and the right auricle. In an ECG obtained the day before death there was a definite depression of the P-Ta intervals in lead II—III (which had also been present though less pronounced in earlier ECG's). LANGENDORF regarded this depression as being caused by the atrial infarct and analogous with the corresponding ECG changes described by LAMBERT. It was however pointed out by CUSHING et al. and by HOLZMANN that P-Ta depression of this degree is also common in proved non coronary cases. Their importance for the diagnosis of atrial infarcts is regarded by these authors as dubious.

The only fairly extensive series of atrial infarcts published hitherto is that of CUSHING, FEIL, STANTON & WARTMAN (1942). They found that the atria were involved in 31 (17 per cent) out of 182 consecutive cases of myocardial infarct. Infarcts were found, in 5 cases in the left, in 27 cases in the right atrium. In 9 cases the infarcts were confined to the walls of the atria. These authors had paid special attention to atrial infarcts during a period of seven years, and thought that their figures reflected approximately the true incidence of the lesion.

The clinical findings in this series were rather inconspicuous. ECG's were available in 23 cases, 17 of which presented some sign of "abnormal auricular mechanism". In 9 cases the abnormal mechanism was represented by auricular fibrillation, in 4 cases by auricular premature beats, in 2 cases by auricular flutter and there was 1 case each of "sinus arrest" and "wandering pacemaker". Such an abnormal auricular mechanism was present only in 8 out of 91 cases of myocardial infarct restricted to the ventricles.

A slight depression of P-Ta-intervals was noted in 5 cases but the authors attached little importance to this finding.

CUSHING et al. also studied the effect of ligation of atrial arteries in 20 dogs. The infarcts obtained in these experiments were as a rule small, multiple, disseminated lesions, differing from the human

infarcts, which were described by these authors as usually massive. Massive infarcts similar to the human ones were observed only in 2 dogs after ligation of the main stem of the right coronary artery. Most of the dogs in these series survived the operation and were observed, in some instances, for several weeks (earlier experimentators had made their observations during the operation only).

The ECG findings after experimental infarction of the atria are described by these authors as rather similar to the clinical observations, at least in the respect that only non-specific disturbances of auricular mechanism appeared. There was a nodal rhythm in 7 cases, wandering pacemaker in 4 cases, auricular flutter in 1 case and more complex disturbances of the atrial rhythm in some other cases. Slight depressions of P-Ta intervals (in which leads?) were observed in 4 cases, and "transient increase in amplitude and contour of the P-waves" in 11 cases. The authors point out, that auricular fibrillation never occurred in any of the cases of experimental atrial infarct, it may be added, that the same observation was made in all previous experimental studies also.

Other observations of these authors will be commented on later in the present study.

A series of 3 right atrial infarcts was presented by YOUNG and KOENIG 1944. There were actual ECGs in two of these cases. The authors think that depressed P-Ta's may be present in lead II---III in one case, but admit that the ECG is technically too unsatisfactory to permit definite conclusions. In the other case they call attention to a marked "dome-shaped" elevation of the P-Ta in lead III, which unquestionably has a very "coronary" appearance. The present writer finds the rest of this ECG a little puzzling at the first glance and is -- after closer scrutiny -- inclined to assume the presence of an auricular flutter with a 2:1 A-V block. This interpretation accounts well for the dome-shaped P-Ta's.

The author has not seen the report of LOPEZ-BRENEZ, VINAMOS and PLEGUEZUELO (1947) in the original. According to a reference in *Excerpta Medica* they describe a case of right atrial infarction with mural thrombosis. There was no ECG in this case.

Recently SKILLER & WEISSEL (1947) described a case with grave coronary sclerosis and multiple ventricular infarcts of varying age in which there was an infarct scar in the lateral part of the right atrium. The patient was a man of 50 with hypertension, who had

suffered for 4 years from anginal pains and died with the clinical picture of an intractable congestive failure. Before death a diphasic P-wave in lead III had been registered in several ECG's. The rather sharp, negative final deflection of this P-wave is regarded by these authors as a  $Q_a$  wave, analogous with the "coronary" Q-wave and referred to the infarct found at autopsy. However, the present writer cannot understand why this deflection, occurring at the end of the P-wave, should be labelled a Q-wave. In the present author's opinion it corresponds to the negative phase that may be found normally at the end of the  $P_{III}$  (LEPESCHKIN), related by some authors to the activation of left atrial structures. If this deflection has anything to do with the atrial infarct observed, it is at any rate of little diagnostic value as similar deflections are often seen in cases in which there is no reason to suspect the presence of atrial infarction.

Finally in some other studies observations of atrial infarcts are mentioned en passant. Thus v. GLAHN found 3 cases of atrial infarcts in his total infarct material. Among 287 cases of myocardial infarcts BEAN found 3 in which the atria were involved and recently HELLERSTEIN & WARTMAN noted 17 atrial infarcts, 13 of which rightsided and 4 leftsided, in a total material of 184 myocardial infarcts.

**b. Some ECG findings, which have been related to atrial infarction, in cases without autopsy control.**

Quite a lot of observations regarding the atrial ECG in cases of coronary heart disease have been reported in literature *without reference to definite autopsy findings*. Many of them have been cursorily mentioned in studies mainly concerned with quite different subjects and it is impossible to survey them completely here. Some such observations will however be mentioned and commented on.

It was noted by MASTER in 1932, that an increase in the amplitude of the P-waves is a common (though transient) finding during the acute stages of ventricular infarcts. He observed such an increase in 32 out of 40 cases. It was usually most obvious in leads I and II and MASTER thought the cause to be a dilation of the left atrium, due to an acute failure of the left ventricle. Similar observations were made by BLOOM & GILBERT (1942), who accepted the explanation given by MASTER and thought that the phenomenon indicated

a bad prognosis. Very high P-waves were observed in some cases of ventricular infarct by HAUSS (1935) who does not comment the finding. The huge P-waves in some cases reported by OETTEL (1941) were however interpreted by him as being possibly signs of atrial infarction. In support of this view he mentioned that in one of the cases there was an occluding thrombus in the circumflex branch of the left coronary artery. No direct proofs of atrial wall lesions are however to be found in the case reports of this author.

UHLENBRUCK (1940) devotes a chapter in his book to the question of atrial infarcts. He exemplifies the condition with a case of his own, in which there was no autopsy, but a peculiar ECG picture which the author found highly suggestive of atrial infarct. It has later been pointed out by LEPESCHKIN (1942) and ÖHNELL (1945), that this ECG is a typical representative of the WOLFF-PARKINSON-WHITE ("WPW") type of ECG. As a matter of fact, UHLENBRUCK had suspected a "bundle of Kent" but rejected this theory when the condition proved to be transitory.

Another case with a WPW ECG has been published by LACHMANN (1943), who also assumed this ECG to indicate atrial infarction. In this case the typical ECG had been present for several years and there was also the typical WPW history of habitual attacks of tachycardia. There was no autopsy in this case either.

Although there can be no dispute about the ECG diagnosis in these cases, some further discussion of the matter is necessary owing to the fact that the genesis of the WPW syndrome cannot be regarded as definitely settled.

The wellknown WPW ECG is characterized by a short P—Q interval (as a rule below 0.12 sec.), QRS-complexes of unusual breadth, due to the occurrence in their initial parts of an abnormal additional deflection (the  $\Delta$  wave of Belgian authors) and finally, more or less pronounced abnormalities also in the S—T and T segments of the ECG. Another definition implies, that the QRS complex is broadened on the cost of the P—Q interval by means of an extra deflection (the  $\Delta$  wave).

It seems to be widely accepted that the cause of the WPW-pattern is a *premature activation* of some part of the ventricular myocardium, (preceding its *normal* activation, mediated by the ordinary A—V pathways). The phenomenon has been termed "preexcitation" by ÖHNELL. Such a premature transmission of the atrial impulse to the ventricles could be realized through extra-nodal muscular bridges between the atria and the ventricles. As a matter of fact such muscular bridges have been demonstrated post mortem in some WPW cases (WOOD, WOLPERTH & GECKELER (1943) and ÖHNELL (1945)).

On the other hand no extra A—V-connections were found in other WPW cases, and nothing is known about their possible occurrence in cases with a

normal ECG. Several authors assume (ÖHNELL, HOLZMANN) that the WPW ECG may be the consequence of acquired myocardial lesions, notably of the *ventricular myocardium*. It has been suggested that the premature activation might in such cases be mediated by a hyperirritable focus in the ventricular wall from which the pre-excitation is released by the mechanical stimulus of atrial systole.

Returning to the views of UHLENBRUCK, we find that he regards the  $\Delta$ -wave as an exaggerated Ta wave (positive in leads II and III) which might indicate an atrial infarct. Recently v. BOGAERT & v. GENABEEK (1947) attempted a similar explanation of the occurrence of WPW ECG's in some cases of coronary heart disease. Some other cases have been published during recent years, in which there was a clinical picture of myocardial infarction and a typical WPW ECG (ZOLL & SACHS (1945) GOLDBLOOM & DUMANIS (1946)). However it has never been proved that such cases represent more than a random coincidence of two not very exceptional conditions. Some years ago the present author made a thorough examination (serial sectioning of the annulus fibrosus and adjacent atrial and ventricular myocardium) of the heart of a case with advanced coronary sclerosis and WPW ECG, without finding a trace of "coronary" lesions in the atria (SÖDERSTRÖM 1946).

The assumption that a WPW ECG may indicate atrial infarction is at present a pure hypothesis; the present author considers further discussion of this matter to be of little advantage until atrial infarction has been demonstrated post mortem in such a case.

In a previous paper (1943) the present author pointed out the occurrence of short P-Q-intervals in cases of myocardial (ventricular) infarct cases in which there was no WPW type of ventricular ECG (no  $\Delta$  wave). The author had some reason to believe that such a shortening of the P-Q interval might be the consequence of atrial infarction. A further discussion of this matter is to be found in chapter VIII.

In a case of myocardial infarct observed by SCHERF & SIEDECK (1934) there was a double P-wave and the distance between the two P-deflections increased during the course of the disease from 0.24 to 0.40 sec. Autopsy showed a thrombosis of the left coronary artery before its bifurcation. The authors assumed the case to be one of interatrial block caused by an atrial infarct (no atrial infarct was however actually noted). Another case of double P-waves after myocardial infarction has been published by MILLER & PERELMANN (1946). They regarded the second P as a positive Ta, indicating atrial "coronary" disease. The distance between the two P-deflections in this case is about 0.06 sec., the present author finds it impro-

bable that the second deflection should be a Ta, occurring after such a short interval. There was no autopsy. Interesting in this connection are the observations of CONDORELLI (1929) who saw inter-atrial block develop in dogs after ligation of the interventricular branch of the left coronary artery (see p. 31).

It may finally be mentioned that SPURHLER (1938) found an elevation of the P-Ta level in the esophageal lead in some cases with coronary heart disease. The significance of this observation must be regarded as rather uncertain.

It can be generally stated that the observations mentioned in which there was no autopsy evidence of atrial lesions, have contributed but little to the knowledge of ECG signs in coronary disease of the atria.

### c. A discussion of the observations in the literature regarding atrial infarction.

Altogether 66 cases of atrial infarcts have thus been at least mentioned in literature up to the present, 52 right, 10 left and 1 bilateral atrial infarcts and 6 cases without specification as to site. Further data are available only in 43 of these cases. Not much can however be concluded from these observations. Taken together with the results of the experimental investigations they do however permit some general conclusions to be drawn about our present knowledge of atrial infarcts.

There seem to be good reasons for the assumption of CUSHING et al., that atrial infarcts are overlooked rather than uncommon. These authors attribute the high incidence of atrial infarcts in their material exclusively to the fact that they actually searched for atrial lesions. It is, however, not quite clear from the study of CUSHING et al. why atrial infarcts are liable to escape attention. They state that "the gross recognition may be difficult" but also that "the gross appearance is common to that seen in ventricular infarcts". The gross recognition of ventricular infarcts is, however, not considered to be very difficult.

A closer study of the reports in the literature made the present author believe, that in most cases it was not the gross appearance of the *myocardium* that awoke the suspicion of atrial infarction but rather some typical complications viz.:

1. Mural thrombosis. This was present in 26 out of the 31 cases of CUSHING et al. who stressed the importance of mural thrombosis as an indicator of atrial infarcts. These authors (legend to fig. 3 in their paper) call attention to a typical dusky purple discoloration of infarcted atria. This description (in the present author's experience) must however be said to apply generally to the external appearance of thrombosed right atria whether there are infarcts in the wall or not.

2. Rupture of the atrial wall. Although of rare occurrence on the whole, it led to the detection of atrial infarcts in 3 of the cases reported in the literature.

3. Subpericardial hemorrhage, which seem to have been the principal gross sign in 3 cases.

It is easy to understand that such secondary consequences of atrial infarcts must attract more attention in routine autopsies than possible alterations in the thin atrial myocardium itself. Histological confirmation of the myocardial lesion was evidently regarded as necessary by CUSHING et al. Not all of the other authors comment directly on the histological findings in their cases.

In general little attention has been paid in literature to the histological picture of atrial infarcts. The present author will return to this matter later on in this study.

Another remarkable fact, that will be left for discussion in another chapter (chapter VIII) is the much higher incidence of infarcts in the right than in the left atrium.

A survey of the signs during life (mainly ECG findings) which have been regarded in the literature as being related to atrial infarcts, is not very encouraging with regard to the possibility of an ante mortem diagnosis.

Naturally, the attention of several previous authors has been concentrated to the *Ta-waves* (*P-Ta intervals*) with the expectation of finding changes analogous to the well known changes in the *T-waves* (*S-T intervals*) following ventricular infarcts. It must also be admitted that three of the experimental investigations (those of LAMBERT, ABRAMSON et al. and SANDERS) all indicate, that typical displacements of the *P-Ta* levels are to be expected in atrial infarcts. According to these authors a depression of the *P-Ta* in leads II—III was common to atrial lesions of all sites. In left atrial lesions ABRAMSON et al. and SANDERS found in addition an eleva-

tion of the P-Ta in lead I. Observations of depressed P-Ta's following experimental atrial lesions are also cursorily mentioned by CONDORELLI (1932) and UNGHVARY (1941). On the other hand CUSHING et al. found slightly depressed P-Ta levels in only a few of their experiments, and do not seem to regard them as significant. It is also important to note that the investigations of ABRAMSON et al. and SANDERS are concerned with direct destruction of the myocardium, which cannot be directly compared with "coronary" atrial lesions. It is only the experiments of LAMBERT and of CUSHING et al. which really reproduce the effect of impaired coronary blood supply to the atria, but their results do not agree. The different results may be due to the fact that different experimental animals were used in these two investigations.

Clinical observations corresponding to the experimental findings mentioned are scanty and not very convincing. Only in the case reported by LANGENDORF there was a *definite depression in the P-Ta levels* of leads II and III. It has, however, already been mentioned that the findings in this case were not accepted as significant by CUSHING et al. and HOLZMANN. The elevated P-Ta's in one of the cases reported by YOUNG & KOENIG have also been commented on earlier in this chapter. The present author feels justified in stating that among the 31 cases of atrial infarct hitherto published in which ECG's were available there is not one in which displacements of the P-Ta's can be said to have had any definite significance.

An *atrial analogy to the deep Q wave* in the ECG of ventricular infarction was noted in some of the experiments of SANDERS. No ECG's were reproduced by this author to demonstrate the appearance of such atrial Qa-waves. The assumed Qa-wave in the case reported by SEILLER & WEISSEL has already been discussed.

Definite changes in the atrial ECG analogous with the typical signs of ventricular infarcts, can therefore not be common after atrial infarction in man.

It was stated by CUSHING et al. that *general abnormalities of the auricular mechanism* represent the most reliable clue to the diagnosis of atrial infarcts. This statement may seem safe, but it must be admitted, that the inconstant observation of auricular fibrillation or premature beats does not represent a clue of great diagnostic value.

No other clinical signs of interest for the diagnosis of atrial in-



farcts have been observed. All cases have been published as autopsy cases, consequently little can be said about the *clinical course* and the *prognosis* — with the exception of the three cases of rupture of the atrial wall in which the atrial infarct must be regarded as the immediate cause of death.

As a summary of the observations in literature it can thus be stated:

1. That infarcts of the atria of the heart are exceptional findings according to most authors but of rather common occurrence according to CUSHING et al.
2. That there is a much higher incidence of infarcts in the wall of the right atrium than in that of the left atrium.
3. That the relative order of size and the morphological picture of atrial infarcts are very incompletely known.
4. That practically nothing is known with certainty about clinical manifestations of "coronary" lesions in the atrial walls.

## CHAPTER III.

### Material and methods.

#### a. Material.

1. The author's main material, to be labelled *series A*, consists of 192 autopsy cases with mural thrombi in the cardiac atria, which were examined for the presence of lesions in the heart wall beneath the thrombi. Of the cases 86 were obtained from the University Institute of Pathology in Uppsala, 66 from the Departments of Pathology, Sahlgrenska Sjukhuset, and Vasa sjukhus (Gothenburg), 30 from the Department of Pathology, S:t Görans sjukhus (Stockholm), 6 from the Department of Pathology, Sabbatsbergs sjukhus (Stockholm) and 4 from the University Institute of Pathology in Lund. The material was collected during the years 1944—1948.

The author personally examined the unprepared hearts of 128 cases. The hearts of the remaining 64 cases had been fixed in 10 per cent formalin and sent to the author from other pathological institutes.

Histological examination of the thrombi and of the atrial wall beneath them was performed in all cases. The author paid special

attention to the occurrence of myocardial infarcts, to be regarded as the main object of this investigation. Other pathological findings were also registered, however, and in addition, due attention was paid to the general structure and the development of the thrombi themselves.

A varying number of blocks were taken from the thrombosed part of the atria, in many cases blocks were taken also from parts of the atria which were free of thrombi. In the first 47 cases blocks were always taken from both the right and the left auricular appendage irrespectively of whether they contained thrombi or not.

All but 4 cases had before death been treated in the hospitals to which are attached the pathological departments mentioned above. The clinical findings in the cases were registered from the routine hospital records. Special clinical examination for the presence of atrial disease had been performed in none of the cases.

The cases of series A were numbered from 1 to 200. The numbers of the autopsy protocols and the hospital records regarding these cases have been registered together with the main clinical and post-mortem findings in a card-index which will be deposited in University Institute of Pathology in Uppsala. Some general data regarding the material is to be found in tables 6 and 7. Some clinical and anatomical data regarding the cases with atrial infarcts have been tabulated in table 8.

2. Some special questions were studied in special materials, which will be briefly accounted for here.

The material of series A proved to be too small and too heterogeneous to form a convenient basis for a discussion of the general frequency and distribution of atrial thrombi. In order to get a satisfactory material for this purpose, the author has registered from the routine autopsy records all cases with *mural heart thrombi* and all cases in which *heart disease* could be regarded as a *main cause of death*, that had been observed during the years 1936—1945 in the University Institute of Pathology in Uppsala. This material will be referred to as *series B*.

It was, naturally, often difficult to decide if heart disease should be regarded as a main cause of death or not and the question had often to be decided in a rather arbitrary way. The author regarded the clinical picture as decisive if the anatomical findings gave cause for doubts. The most important groups of doubtful cases is formed

by cases with advanced coronary sclerosis, which had presented no conspicuous signs of this disease during life and which had been treated during life under various diagnoses such as fractura colli femoris, prostatic hypertrophy etc. They were included in the group of cases dying in heart disease only if recent myocardial infarcts or extensive scars had been registered. Some cases in which the immediate cause of death registered was another (viz. cerebral accidents, pulmonary embolism etc.) but the clinical picture of which had been dominated by direct signs of heart disease have also been registered as dying of heart disease.

Series B consists of 882 cases with heart disease as a main cause of death, collected from a total number of 4998 autopsy records. Mural heart thrombi were found in 389 cases, in 14 of these cases heart disease was *not* regarded as a main cause of death. For a closer definition of the material the reader is referred to tables 1—5.

3. In order to find typical changes in the atrial ECG during the course of ventricular infarcts the author has studied the ECG's from 268 cases of myocardial (ventricular) infarction which had been treated in Akademiska sjukhuset, Uppsala, during the years 1936—1947 and from 104 such cases treated in the 2nd medical department (Head: Professor Gustav Nylin) of Sabbatsbergs sjukhus, Stockholm, during the years 1943—1946. This study was not very fruitful, and systematic changes in the atrial ECG of general interest were observed only in a few cases. No complete account for the results of this investigation will be given in this study. Isolated observations in this material (*series C*) will however be described in the following chapters.

4. In addition to the cases in series A, histological examination for atrial infarcts was performed in about 50 cases without mural thrombi when other signs justified the suspicion of atrial infarction. In 7 of these cases minor lesions were found but only in one case sufficiently extensive lesions were present to justify the label "atrial infarction" according to the definitions in chapter VIII.

Some smaller, additional materials, which were collected in order to elucidate various other questions will be accounted for separately in the following chapters.

5. The grouping of the material according to diagnosis in some of the tables should be commented on here. Most of the cases could be classified as belonging to one of three large diagnosis groups:

1. rheumatic heart disease, 2. coronary heart disease and 3. hypertensive heart disease. The remaining cases were usually placed in a fourth group of miscellaneous diagnoses (congenital heart disease, chronic "cor pulmonale", septic myocarditis etc.). The demarcation between the groups of coronary heart disease and hypertensive heart disease is however difficult to define. Most cases of hypertensive heart disease have also a coronary sclerosis and the fall in the blood pressure due to acute myocardial infarction often conceals the presence of a hypertensive heart disease. In order not to complicate matters the author introduced the diagnosis group *coronary sclerosis-hypertensive heart disease*, including all cases of hypertensive heart disease with considerable coronary arterial sclerosis, recent myocardial infarcts or extensive myocardial scars. The remaining cases of hypertensive heart disease without signs of "coronary" heart disease form the diagnostic group "(uncomplicated) hypertensive heart disease".

Only cases with rheumatic *valvular* disease were placed in the group of rheumatic heart disease.

#### b. Histotechnic remarks.

The material for histological examination was usually fixed in 10 per cent formalin and embedded in paraffin. For the material to be stained for glycogen absolute alcohol was used as a routine fixation fluid. This material was embedded in celloidin and paraffine.

As a rule sections from at least 2 levels in the same block were examined. Serial sectioning was not performed.

Sections were stained according to VAN GIESON in all cases, in some cases of series A additional sections were stained with hematoxylin and eosin. In a few cases sections were stained for elastin and fibrin (WEIGERT's fibrin stain) and for amyloid (methyl violet). Staining for glycogen according to BEST was performed in sections from 22 cases of series A and in sections of atrial and ventricular myocardium from 46 other cases, 9 of which had ventricular infarcts. In 39 cases of series A sections were stained for fat with Sudan III.

Some remarks should be made to the demonstration of glycogen in the myocardium according to BEST. It is well-known, that this staining method is not absolutely specific for glycogen. Some other substances (mucin, cartilage, granules of the *mast-cells* etc.) may

also take the same red colour as glycogen. The present author has actually seen some examples of "pseudoglycogen" in sections from the heart. In these cases a fibrillary substance in the endocardium and in the intima of some vessels had taken on a bright, red colour. It could however not be mistaken for the usually granular glycogen which occurs within the muscle fibres. If there is any doubt the identity of the substance may be established by means of the saliva test (see ROMEIS), which was performed by the author in some of the cases first examined. Otherwise the glycogen is easily identified by the effects of the "Alkoholflucht des Glycogens" (ROMEIS). In alcohol-fixed preparations the glycogen has a tendency to accumulate in the parts of the cells most distant from the surface, from which the alcohol penetrates into the piece of tissue. In fibres cut transversely the intracellular glycogen is thus usually distributed in crescents with the concavity facing the nearest free surface of the preparation (fig. s. 22, 24).

### c. Electrocardiograms.

Most ECG's reproduced or referred to in this study are clinical, routine ECG's taken with an amplifier apparatus for three or four simultaneous leads (system ELMQUIST). Some ECG's were taken with an amplifier apparatus for consecutive leads (Siemens).

The three conventional extremity leads, and in most cases also a chest lead (IVR) were registered. All ECG's have been calibrated to make 1 cm equal to 1 mV.

The U-P interval was regarded as representing the isoelectric level measurements of the amplitudes of the P-waves or the displacements of the P-Ta intervals. If no reliable U-P interval was present (and this was the rule rather than the exception; see p. 36) no attempt was made to measure these amplitudes.

The author has used the term P-Q-interval (or P-Q-time) for the time interval between the beginning of the P-wave and the beginning of the ventricular complexes. The term of P-Ta interval or P-Ta level has been used to signify the level of the part of the P-Ta interval which is visible before the ventricular complex.

\*

Statistical data given in this study have been calculated according to accepted methods.

## CHAPTER IV.

## Some anatomical and functional data regarding the atria of the heart.

This chapter is not intended to give a complete survey of the anatomy and the physiology of the atria of the heart. For full accounts the reader is referred to the current manuals. The author's purpose is to point out some facts — for the most part little known — which are of importance for the understanding of observations discussed in the following chapter, and to define some terms to be used in this discussion.

The descriptions are based partly on the statements of other authors and partly on observations by the present author. It must be emphasized at this point, that the contributions of the present author are not the result of systematic observations on a substantially normal material, but rather impressions formed and gradually confirmed during the study of numerous more or less abnormal hearts at autopsy during the last 5 years. In order to elucidate particular questions, the author investigated from time to time presumably normal specimens which were however studied from such varying standpoints that they cannot be tabulated.

## a. Some remarks to the general anatomy of the atria.

In general there are obvious analogies in the gross design of the left and the right atrium. (Fig. 1.) In both there is a trabeculated part mainly occupying the ventral part of the atrium and furnished with an auricular appendage, projecting medially. The terms just used will be adopted in the following discussion to avoid the somewhat ambiguous conception of "auricle". In both there is also a smoothwalled part mainly occupying the dorsal part of the atria and deriving, at least partially, in the right atrium from the sinus venosus and in the left atrium from the primordial pulmonary vein(s).

Particularly interesting facts from the author's point of view are however to be derived from a study of the *difference* between the right and the left atrium which is particularly conspicuous as regards the interior architecture.

The inner design of the *left* atrium (Figs 1, 2) is rather simple.

The trabeculated part occupies but a small portion of the total wall surface, a relation that may be extremely exaggerated in dilated specimens. In the left atrium the conceptions of auricular appendage and trabeculated part are more or less synonymous. The trabeculae carneae are few, coarse and short and arranged to shut off along the margins of the appendage a row of rather simple caverns with narrow openings into the main atrial lumen. The rows of such caverns visible in longitudinal sections of the left auricular appendage often form a picture suggestive of a snail's shell. There often is no very sharp borderline between the trabeculated and the smooth-walled parts of the left atrium. The inner surface of the smooth-walled part is as a rule devoid of peculiar structures of sufficient importance for this study to merit a special description.

The inner structure of the *right* atrium is more complicated. The trabeculated part here occupies at least half the inner surface. The auricular appendage is more or less filled up by a spongy mass of fine, richly anastomotic trabeculae. Laterally the trabeculae are coarser and arranged parallel to one another, forming the typical long colonnade of *musculi pectinati* (older terminology). Dorsally the margins of the trabeculated part converge acutely to form a narrow band of trabeculated wall, passing between the orifice of the caudal vena cava and the tricuspid ostium, to fade out in the region of the orifice of the sinus coronarius (fig. 1). The smooth-walled part of the right atrium is thus divided into two parts, one occupying most of the dorsal and the septal wall, the sinus part of the atrium, and another surrounding the tricuspid ostium and termed by the present author the tricuspid infundibulum (fig. 1, 3). The trabeculated part is sharply delimited from the rest of the atrium by the ridge of *erista terminalis*, the cranial part of which at least is very prominent, and by the cranial border of the tricuspid infundibulum. Medially there is a more diffuse transition to the smooth aortic wall of the right atrium.

There exists furthermore in the right atrium a very peculiar system of recesses, which is important for the distribution of mural thrombi. In this study these recesses will be referred to as auricular sinus. They are filled out with the same spongy trabecular masses as the right auricular appendage, and may be regarded as continuations of the sinuous lumen of the auricular appendage, *within the walls of the main part of the atrium* (figs 3, 4, 5, 6). The most important

of these auricular sinuses undermines the tricuspid infundibulum and occupies (in sections perpendicular to anulus fibrosus) a triangular space between the tricuspid infundibulum, the coronary groove and the external wall of the atrium (fig. 3). This auricular sinus may form an uninterrupted recess from the auricular appendage, along the coronary groove to the orifice of sinus coronarius. Dorsally it may attain considerable dimensions, forming a projection on the external contour of the atrium (subeustachian sinus (KEITH) auricula posterior (HIS)). A similar auricular sinus undermines crista terminalis to a varying distance laterally from the auricular appendage. It widens medially to form a large recess, attached to the ventral wall of vena cava cranialis. This recess, which is a frequent site of mural thrombi, will be called in this study antrum atrii dextri (fig. 4 A).

The lumen of the auricular sinus consists of a complex system of intertrabecular spaces, which are normally extremely narrow and easily overlooked (fig. 5). When filled out with thrombus substance, however, they are very plainly visible (fig. 6). The microscopical picture may be very similar to that of the "spongy" myocardium of fetal hearts. The auricular sinuses open into the main lumen between the pectinate muscles.

It may be mentioned that the extension of the auricular sinus may vary within wide limits. The subeustachian sinus of KEITH often forms an independent recess, not continuous with the other sinus systems.

Some other anatomical facts may be briefly mentioned.

The *endocardium* of the left atrium, and of the smooth-walled part of the right atrium is rather thick, containing several elastic lamellae and a more or less conspicuous layer of smooth muscle cells. The normal endocardium of the left atrium has been studied by several authors but especially by GROSS (1935), who states, that the endocardium is thicker and the smooth muscle layer more conspicuous in aged subjects.

In the trabeculated part of the right atrium the endocardium is extremely thin (5—20  $\mu$ ) and consists only of the endothelial layer seemingly placed directly on the perimysial connective tissue of the myocardium.

The *myocardium* in the smooth-walled parts of the atria forms a simple muscular layer, about 1—3 mm thick. It seems to be more



massive in some places, e. g. the interatrial septum and the so called Torus Loweri (a myocardial ridge, not very prominent in human hearts, passing from the mid part of the crista terminalis to the region of sinus coronarius. It is important as a short communicating path between the sinus and the A-V nodes). Histologically these thicker parts of the atrial wall usually consist of rather scanty myocardial fibres embedded in fat tissue.

In the trabeculated parts of the atria no figures can be given for the thickness of the atrial wall. Specially in the right atrium there is in some places no myocardial tissue between the endocardium and the pericardium. The thickness of the trabeculae varies within wide limits. With the exception of the more massive structure of the crista terminalis it is difficult — even in the case of hypertrophy — to find a spot in the trabeculated atrial wall more distant from the nearest endocardial or pericardial surface than 1 mm.

#### b. The nutrition of the atrial walls.

Thorough studies of the atrial *arteries* are to be found in the works of GROSS (1921), CRAINCICANU (1922) and SPALTEHOLZ (1924). The conformity between the statements of the different authors is in most respects fairly good. In this survey the author will follow the description of SPALTEHOLZ.

SPALTEHOLZ describes 3 main atrial branches from the right coronary artery and 3 from the circumflex branch of the left coronary artery. The branches from the right coronary artery have been called by SPALTEHOLZ ramus atrialis dexter anterior, *intermedius* and posterior, the ones from the left coronary artery ramus atrialis sinister anterior, *intermedius* and posterior. It may be mentioned that the symmetrical pair of anterior (ventral) branches which as a rule are important originate from early segments of the main coronary arteries. A description of the other branches is of little purpose as their relative order of size and their nutrition regions may vary within very wide limits. In general it seems to be true, that the right atrium is nourished from the right coronary and the left atrium from the left coronary, but there are numerous exceptions to this rule. According to most authors, that region of the right atrium, which surrounds the orifice of the vena cava cranialis, is in particular nourished from the left coronary artery in a considerable number of cases.

The region mentioned has indeed been the object of much discussion in regard to its arterial blood supply. KEITH & FLACK (1907) being especially interested in the very constant artery of the sinus node, argued for the constant existence in this region of an arterial circle receiving its blood from both the right and the left coronary artery. The investigations of GROSS and SPALTEHOLZ were not in favour of this assumption. SPALTEHOLZ found a complete arterial circle in only 6 cases out of 18 and only in one of them was the circle formed by branches from both the right and the left coronary artery.

Special interest has been devoted by different authors to some individual atrial arteries of remarkable constancy. The artery of the sinus node has already been mentioned. It seems to be identical with (or sometimes a branch from) the ramus cristae terminalis of SPALTEHOLZ, which has a very constant course along the cranial half of the sulcus terminalis. This artery may originate from almost any one of the atrial branches mentioned. SPALTEHOLZ regards it as a remnant of an incomplete arterial ring, present in the "coronary" groove between the sinus venosus and the atrium proper in some lower vertebrates. A similar rôle is assigned by this author to a "transverse artery of the left atrium", passing along the Taenia terminalis sinistra of KEITH to the region of vena cava cranialis and carrying, in most instances, the supply of blood from the left coronary to this region. Finally KUGEL (1927) describes an artery under the name of arteria auricularis anastomotica magna which takes its origin from the first segment of the left coronary (from the ramus atrialis sinister anterior?) and passes backwards through the atrial septum, to anastomose in the dorsal wall with branches from the right coronary. Possibly this artery corresponds to the arteria interauricularis recurrens described by CONDORELLI (1928) in the heart of the dog. CONDORELLI ascribes to the existence of this artery the appearance of inter-atrial block after ligation of the ramus interventricularis of the left coronary in his experiments.

SPALTEHOLZ states that in his material interarterial anastomoses were common in the left but exceptional in the right atrium. He regards this fact as rather unexpected in view of his experience of anastomoses in the ventricular walls.

The observations on the branches of *non-coronary arteries* taking part in the arterial supply of the atria are of special interest. KOCH (1909) and BELOU (1934) have seen the sinus artery in human hearts originate from the left bronchial artery and in 3 of his cases SPALTEHOLZ observed rather important extra coronary branches ramifying in the atrial walls. In their attempts to stop the blood supply to the sinus node in dogs, ROTHBERGER & SCHERF were often inconvenienced by such extra coronary branches. Finally it was observed

by WEARN et al. (1932) using injected preparations that normally numerous and sometimes rather large anastomoses to extracoronary artery systems are regularly present in the walls of the venae cavae and venae pulmonales.

The *atrial veins* and especially the so called *venae minimae* (Thebesii) deserve a special discussion because of their possible importance for a "retrograde" supply of blood to the myocardium when the coronary arteries have become occluded. Numerous small venous vessels are known to open into the lumen especially of the right atrium. Some authors maintain, that there is an essential difference between the more differentiated coronary veins and the more simply structured *venae minimae* (or *vasa minima* to use the term suggested by BATSON and BELLET (1930)), both opening directly to the heart lumen. A short survey of the development of the myocardial vessels during fetal life may contribute to the understanding of these relations.

In early embryonal stages, the myocardial wall of the whole heart has a spongy appearance and is penetrated throughout by a system of irregular spaces and channels communicating with the main lumen. In these stages the myocardium must be nourished by a "sinusoidal circulation" (LEWIS (1904)) through these cavities. The sinusoidal system disappears gradually during fetal life and its functions are taken over by the developing coronary arteries and veins. According to GRANT (1926) the regressing sinusoidal vessels are joined by the ingrowing coronary arteries and veins; *some of them remain as venae minimae*.

It has been shown, that in adults *venae minimae* may communicate with capillary nets (GRANT & VIKO (1929)), with coronary veins (THEBESIIUS (1708)) and with coronary arteries (ABERNATHY 1798). The last type of connections have been studied especially by WEARN and his collaborators. WEARN, METTIER, KLUMPP & ZIESCHE (1933) saw arteries of up to a diameter of 0.4 mm open directly into the lumen of the heart.

The venous drainage of the right atrium seems to be performed through short venous channels directly to the lumen of this chamber. It was pointed out by GERAUDEL (cit. after BLAIR and DAVIS) that no large vein can be seen to follow the sinus artery. In the present author's opinion the same can be said about many other arteries in the right atrium, in which myocardial veins are not nume-

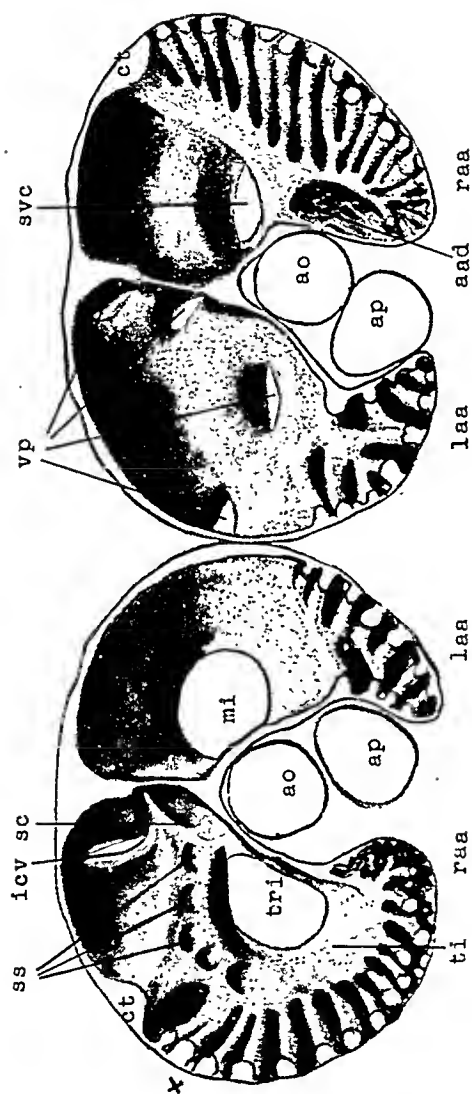


Fig. 1. Inner surface of the atria of the heart in man. Semischematic drawing from a typical normal specimen, fixed in formalin. The contour of the caudal part of crista terminalis is somewhat exaggerated.

The section is made through the largest circumference of the atria, in a plane which is roughly parallel to that of annuli fibrosi. aad "antrum atri dextri", ao aorta, ap pulmonary artery, ct crista terminalis, icv vena cava caudalis, laa left auricular appendage, mi ostium mitrale, raa right auricular appendage, sc sinus coronarius, ss subeustachian sinus (KEITH) or auricula posterior (HIS), svc vena cava cranialis, ti triuspid infundibulum, tri ostium trienspidale. ( $\times 2/3$ ).

AAD



LA



RA

Fig. 2. Inner aspects of the left (LA) and the right (RA) auricular appendages. AAD "antrum atri dextrae". Normal specimens, fixed in formalin. ( $\times 1\frac{1}{2}$ ).

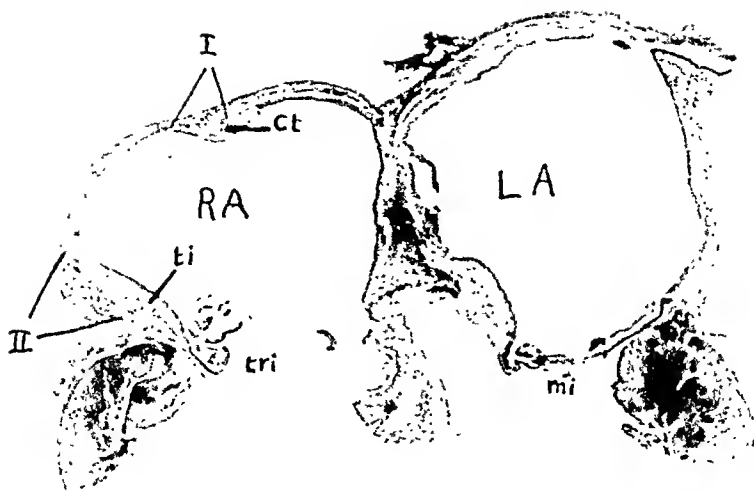


Fig. 3. Section through the largest diameter of the two atria, roughly perpendicular to the plane of the annuli fibrosi.

RA right atrium, LA left atrium, tri and mi tricuspid and mitral ostia, ti tricuspid infundibulum, ct crista terminalis, I and II *auricular sinus underlining crista terminalis and tricuspid infundibulum*. (Normal atria from a child, 2 years old, v. Gleason,  $\times 2.5$ ).

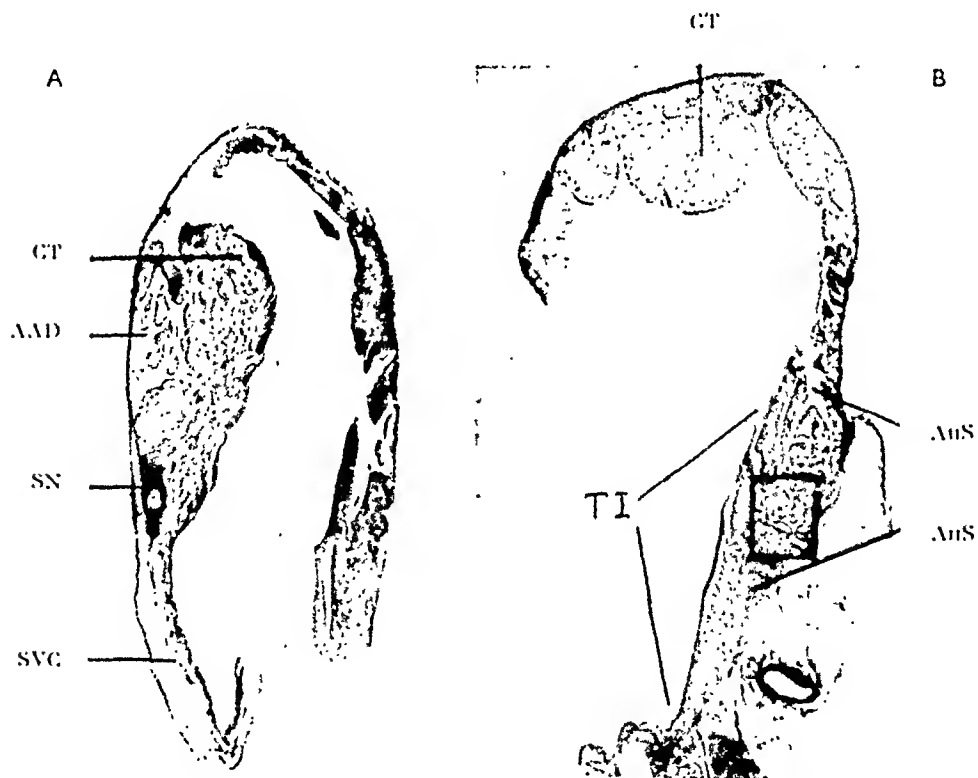


Fig. 4. A. Section through the "antrum atrii dextri" (AAD). B. Section of the lateral wall of the right atrium (position roughly corresponding to x in Fig. 1).

CT crista terminalis, SN sinus node, SVC ventral wall of vena cava cranialis, TI tricuspid infundibulum, Au S auricular sinus. (normal atrium, male 20 years old, v. Gleason,  $\times 3$ ).



Fig. 5. The area within the rectangle outlined in fig. 4 B. "Spongy" structure of the myocardium with a complex system of intertrabecular spaces (v. Gieson,  $\times 30$ ).

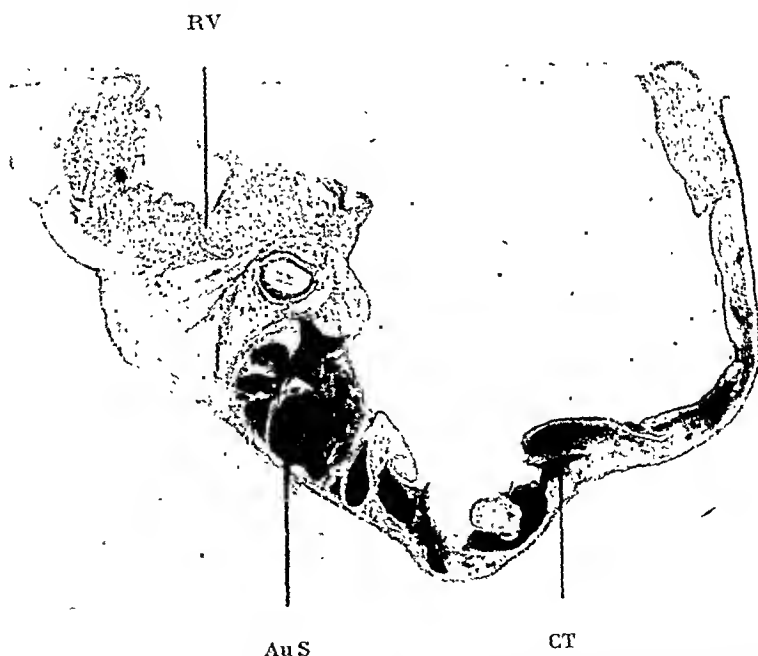


Fig. 6. Right atrium. Infarct in the smooth-walled part, including the crista terminalis (CT). Thrombosis of adjacent auricular sinus (Au S "subeustachian sinus"), the walls of which are histologically intact. RV right ventricle (Ser. A case 153, v. Gieson,  $\times 2 \frac{1}{2}$ ).

rous and if present, are difficult to differentiate from narrow intertrabecular spaces with endocardial covering. Larger veins in the right atrium have been described by some authors but seem not to be very constant.

The venous drainage from the left auricle is very incompletely studied, probably it is mainly directed to the sinus coronarius (the vena obliqua atrii sinistri MARSHALLI is a well-known vessel draining in this direction). Even THEBESIIUS stated that vessels opening directly to the lumen were more scanty in the left than in the right atrium and in the perfusion experiments of LÆNDRUM, KONDO & KATZ only an insignificant amount of fluid found its way to the left atrium.

It is a very old theory, that the nutrition of the myocardium, after the occlusion of a coronary artery, could be maintained by means of a "retrograde" circulation through vessels (venae coronariae, venae minimae), which open directly into the lumen of the heart. The discussion in the literature has mainly been concerned with the question of whether the myocardium of the *left ventricle* could be nourished in this way. There are important reasons for not accepting this possibility; few venae minimae open directly into the lumen of the left ventricle, and during life the systolic pressure within the left ventricle is too high to permit a perfusion with blood from the right heart (see BATSON & BELLET, WIGGERS).

The situation is certainly quite different in the walls of the atria. Even disregarding the possibility of a "retrograde" *circulation*, nutrition directly from the lumen may be of importance for the thin atrial walls. It may be quite impossible to differentiate in microscopical sections venae minimae from the fine intertrabecular spaces which are abundantly present in the auricular appendage or the auricular sinuses. It is however not more difficult to imagine the mechanism of blood supply to the myocardium by the one than by the other of these channels. It may be a simple to and fro movement of the blood, comparable with the mechanism probably present in the fetal heart.

Thus one can certainly assume that the atrial walls, as compared with the ventricular walls, are in a rather favourable position with respect to the chances of alternative blood supply, either by means of interarterial anastomoses along the large veins (venae cavae or venae pulmonales) or by means of nourishment directly from the lumen of the heart.



### c. The specific nodes and the normal atrial ECG.

The general topography of the specific nodes in the wall of the right atrium is too well known to need a special description here.

It may be mentioned, that in the present author's experience, the *sinus node* presents a very typical histological picture. The author consciously tried to make sections of the right atrial wall to pass through the sinus node, in order to facilitate orientation in the slides. The easy recognition of the sinus node depends essentially on the typical combination of certain formal elements: the strong sinus artery, surrounded by the dense fibrous tissue of the node as by an enormous adventitia, in which a high power scrutiny reveals the slender, tortuous fibres of the nodal myocardium. If some element, e. g. the sinus artery, was lacking in its proper place, the author was always in doubt as to whether a suspect tissue was really to be regarded as belonging to the sinus node. When the sinus fibres were leaving the node to ramify between the ordinary myocardial fibres the author was able to recognize them only as long as the continuity with the parent tissues in the node was not broken.

The *A-V node* was searched for in this study only in a restricted number of cases. A structure corresponding to the A-V-bundle was usually found, but the author was never able to find any distinct formation corresponding to the current conception of the A-V node.

The author's experience thus agrees with the opinions of MÖNCKEBERG, MAHAIM, TODD and other authors, when they regard the specific myocardial *fibres* in the human heart as at least very difficult to differentiate morphologically from ordinary fibres. The specific nets of Purkinje-cells recognized by morphological studies in human atria by some authors (THOREL, TANDLER and others) are certainly to be judged with great caution.

The current conception of the activation mechanism within the atria also still seems to be in accordance with the theories of LEWIS. This means, that the impulse, starting in the sinus node, is diffused through the atrial walls, by means of common myocardial fibres. Authors like ROTHBERGER & SCHERF and CONDORELLI (1929) when speaking about conduction pathways in the atria, obviously did not regard them as specific structures, but rather as possible pathways in ordinary myocardium, preferred because of their shortness.

Because of the position of the sinus node there must be an activa-

tion of exclusively *right* atrial structures during a short period in the beginning of atrial systole. The exact duration of this period in man is not known. It is further known, that the left auricular appendage is the last part of the atria to be activated. According to RORNBERGER (1926), LAUFER and others the activation of the left auricular appendage is completed 0.01--0.03 sec. after that of the right auricular appendage. During the rest of the atrial systole right and left atrial structures are activated simultaneously. There is thus little reason for speaking about an interatrial conduction time. For the rest little is known about the time relations of atrial systole. The part of the left atrium reached by the esophageal electrodes is activated 0.02--0.06 sec. after the first signs are seen of right atrial activity, the period of purely right atrial activity is probably somewhat shorter.

*The atrial electrocardiogram* has the same two main constituents as the ventricular one. The P-wave represents the initial complex and there are equivalents for the S-T-interval and the T-wave, commonly termed in the atrial ECG *the P-Ta interval* and *the Ta-wave*.

The P-waves of the limb leads differ from the ventricular initial complexes in their lower amplitudes and their slow curves of increase and regression. These differences are usually attributed to the smaller quantity of active myocardium and to the lack of a special conductive system in the walls of the atria.

The figures given by different authors for the *normal duration of the P-waves* are somewhat varying. The figures of LEPESCHKIN are 0.08--0.12 sec. As a rule the P-waves are shorter and begin later in lead I than in leads II and III. Some authors assume that the P-wave of lead I corresponds to the activity of the left atrium (LEPESCHKIN, § 138). There is at least some reason to believe, that potentials from the right atrium are poorly represented in lead I.

The P-waves are normally positive in leads I and II; in lead III they are sometimes invisible, diphasic or negative. According to LEPESCHKIN the mean *amplitude of the P-waves* in lead I is 0.07 mV, in lead II 0.14 mV and in lead III 0.09 mV. The extreme values for the amplitudes of the P-waves in normal cases are, according to the same author, + 0.5 mV (in lead II) and - 0.44 mV (in lead III). Such extreme values are certainly seldom seen, but it is important to state, that the amplitudes of the P-waves may vary considerably also in

normal cases, in particular under the influence of the autonomic nerve system (NORDENFELT 1941, 1940). Similar experiences have been reported by several other authors. It is at any rate certainly true, that changes in the amplitudes of the P-waves may only with great caution be evaluated as signs of atrial myocardial disease.

The same can be said of the *Ta-wave and the P-Ta interval*. It is only possible to observe this part of the atrial ECG in its entirety in cases of A-V block. In such cases it can be seen to be represented (in the limb leads) by a single, very shallow, negative deflection (sometimes no deflection at all is present). It is important to note that the duration of the P-Ta interval is approximately of the same order of size as that of the Q-T interval, according to MALMSTRÖM between 0.14 and 0.32 sec. in cases with absolute A-V block. This means that normally only a short initial segment of the P-Ta pattern can be observed (between P and Q), the rest being hidden in the ventricular complex.

According to most authors this normally visible part of the P-Ta interval is also as a rule negative. HOLZMANN and LEPESCHKIN state, that its potential is generally opposite to that of the P-wave. In a normal material SHIPLEY & HALLARAN found, that the P-Ta level was usually isoelectrical in lead I and negative in leads II and III. In the limb leads the deviation from the isoelectric line is seldom conspicuous, but definite figures for its normal variation are as yet lacking. According to HAHN & LANGENDORF a depression exceeding 0.05 mV in rest and 0.1 mV after exercise is to be regarded as abnormal. Appreciable *elevations* of the P-Ta level are probably to be regarded as pathological if the P-waves are positive (HOLZMANN).

The present author studied the visible part of the P—Ta interval in 500 non selected ECGs from this clinic: 250 ECGs from patients aged between 26 and 32 years, 250 ECGs from patients aged between 61 and 69 years. Heart disease was probably present in most of these cases at least in the higher age group. The following observations were made:

1. In such routine ECG's it proved impossible to evaluate with any degree of accuracy P—Ta displacements not attaining the value of 0.05 mV. Consequently only displacements reaching or exceeding 0.05 mV were registered.
2. Apparent depressions of the P—Ta were noted in 105/250 ECG's within the older age group and in 145/250 ECGs within the younger age group. Depressions were most often seen in leads II and III or in the chest lead (IVR). Depressed P—Ta's in lead I were observed in only 2 cases. Elevated P—Ta's were observed in 6 ECG's, all in lead I and all occurring in the older age group.

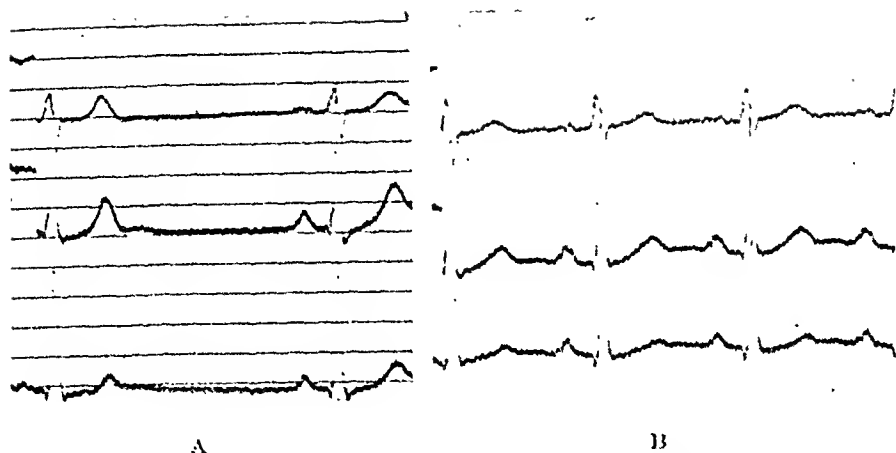


Fig. 7. Consecutive ECG's from a patient without manifest heart disease. Apparent depression of the P-Ta's of leads II and III in B, probably due to an elevation of the T-P intervals by an U-wave (distinctly visible in A).

P-Ta depressions were about as common in otherwise normal ECG's as in pathological ones, elevated P-Ta's were found only in definitely pathological ECGs.

3. A closer scrutiny showed however, that in most cases the P-Ta depressions were possibly only apparent and due to the presence in the T-P interval of a more or less distinct, positive U-wave. This low deflection has a considerable duration — the author found the mean duration in 108 ECGs to be  $0.193 \pm 0.027$  sec. T-P intervals shorter than 0.25 sec. (heart rates above 85/min. approximatively) are thus often completely occupied by an U-wave, which may be rather difficult to discern. At any rate its presence can seldom be excluded with certainty. No correct isoelectric level can be established in such cases, but one is easily misled into constructing false isoelectric levels through the flat tops of the U-waves or through the depressions between T and U. In both cases the P-Ta may seem depressed. Among the 250 ECG's, in which P-Ta depressions were observed, they could be related to a reliable isoelectrical level in only 13 cases. In all other cases the T-P distance was less than 0.25 sec. and more or less obviously occupied by an U-wave. In some cases the combinations a) slow heart rate — distinct U-waves — isoelectric P-Ta's and b) accelerated heart rate — indistinct U-waves — depressed P-Ta's were seen to alternate repeatedly in consecutive ECGs from the same case (figs 7, 8).

Finally, in all the cases with apparent elevation of the P-Ta in lead I the explanation proved to be the presence of an negative U-wave of the type usually following deeply negative T-waves in this lead.

These observations regarding the P-Ta level have made the author agree with HOLZMANN (though for other reasons), when he states, that only extreme displacements of the P-Ta level can be discussed as possible indications of "coronary" atrial lesions.

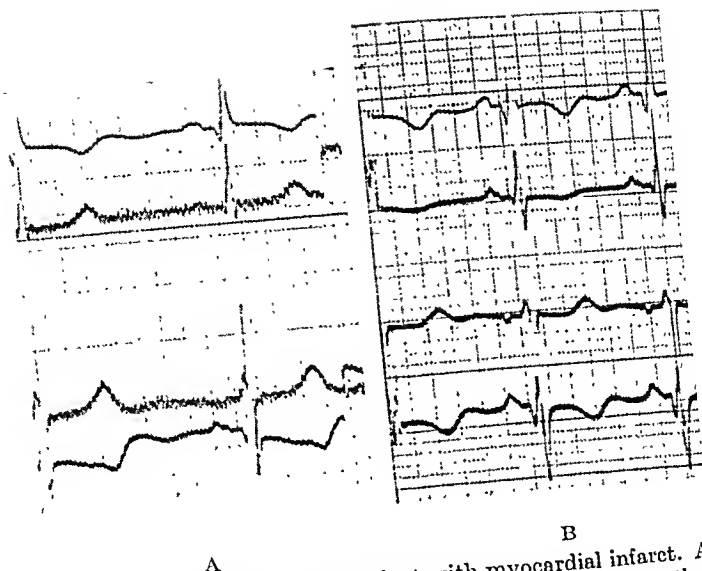


Fig. 8. Consecutive ECG's from a patient with myocardial infarct. A negative U-wave in lead I and IV (distinctly visible in A) accounts for the apparent elevation of the P-Ta's of lead I and IV in B.

*The duration of the P-Q interval* has obviously nothing to do with that of the P-Ta interval. Disturbances of the intra-atrial conduction mechanism may however in some instances also affect the duration of the P-Q interval. A further discussion of this matter is to be found in chapter IX.

It can be generally stated, that the deflections forming the atrial ECG in the limb leads are small, variable and difficult to measure with certainty. If atrial "coronary" disease is reflected in the atrial ECG (of the limb leads) one cannot expect such ECG findings to be very striking, and they are always to be judged with great caution because of the numerous sources of error.

Special chest leads have been suggested by several authors in order to secure a more distinct tracing of the atrial ECG. P-waves of a considerable amplitude and quite distinct Ta-waves may be registered in esophageal leads. However, no reference will be made to such leads in the following chapters and it would consequently be of no purpose to describe in this place the atrial ECG in special leads.

### Summary.

The following viewpoints should be stressed as being of particular importance for the main scope of this study.

The trabeculated part occupies a much larger portion of the inner surface of the right than of the left atrium.

The trabecular branchery of the right auricular appendage has a finer and more complex structure than that of the left auricular appendage. The lumen of the right auricular appendage thus may be characterized as having a "spongy" structure. The same "spongy" branchery of trabeculae is filling up also some long recesses (auricular sinuses), which form continuations of the lumen of the auricular appendage within the walls of the main part of the atrium.

The right atrium is mainly nourished by branches of the right coronary, the left atrium in a corresponding way by branches from the left coronary. Intercoronary anastomoses exist in the atrial walls. Extracoronary arteries have often been observed to take part in the nutrition of the atrial walls. It may be assumed that the possibility of a nutrition by means of such extra-coronary arteries is of greater importance for the atrial than for the ventricular walls.

Nutrition directly from the lumen blood may be of considerable importance for the thin atrial walls. The numerous intertrabecular spaces, vasa minima and short coronary veins present; at least in the right atrium, mean a considerable augmentation of the surface of contact, through which this nourishment (diffusion of oxygen etc.) can take place.

The deflections of the atrial ECG in the clinical standard leads are small, variable and difficult to measure with certainty. Changes in such routine ECGs indicating atrial infarction cannot be expected to be of striking dimensions and are to be judged with great caution because of the many sources of error present.

## CHAPTER V.

### **Mural thrombosis in the atria of the heart.**

The author's interest in atrial mural thrombi was based on the assumption, that they possibly indicate lesions in the atrial wall. It is, however, not generally accepted that wall lesions really represent important causes for the formation of thrombi in the atria. The author thus feels justified in devoting this chapter to a general survey of the etiology and development of such thrombi.

Factors responsible for the formation of intra-vital clots are conventionally divided into 3 main groups, viz. 1. lesions of the vascular wall, 2. circulatory factors (mainly to be identified with a stagnation of the blood) and 3. chemical and morphological changes in the blood, causing an increased tendency for clotting.

Factors belonging to group 3. will be left aside as of little interest for the main scope of the present study. It may be assumed that such factors are not often of decisive importance for the local formation of thrombi in the heart.

The author's attention will instead be concentrated on factors belonging to group 1. In cases of heart disease such factors are however often difficult to isolate from factors belonging to group 2. This very question, whether wall lesions or stagnation factors are the decisive causes for the formation of atrial thrombi, must however be a central matter for discussion in this chapter.

The morphological observations described in this chapter (section b.) were made in the cases of series A. This series is, however, rather heterogenous and cannot be related to a common autopsy material. The discussion regarding the frequency of atrial mural thrombi (in the sections a. and c. of this chapter) will consequently instead be based on figures derived from series B.

#### **a. General conditions for the formation of mural heart thrombi.**

Atrial and ventricular mural thrombi are essentially analogous formations, and it may be assumed, that they have at least some etiological factors in common. The etiology of most ventricular thrombi is a less complicated matter than that of atrial thrombi. Some preliminary viewpoints of interest might thus be expected from a comparison between atrial and ventricular thrombi with respect to frequency and distribution.

Statistical studies of the frequency of mural heart thrombi have previously been published by HARVEY & LEVINE and by GARVIN. Figures from these papers have been tabulated in table 1 together with corresponding figures from the present author's series B. Briefly, it can be concluded from these figures, that mural thrombi are common in the left ventricle and the right atrium and somewhat less common in the left atrium and the right ventricle. The only striking

Table 1. Figures illustrating the frequency and distribution of mural heart thrombi. The figures are taken from the studies of HARVEY & LEVINE (H. L.), GARVIN (G.) and from series B in the present study (S.).

	RA	LA	RV	LV	CT	HD	TA
H. L. ....	60	28	17	59	111		2091
G. ....	125	49	56	156	264	771	6285
S. ....	162	137	57	168	389	882	4998

Cases with thrombi in the right atrium = RA, in the left atrium = LA, in the right ventricle = RV and in the left ventricle = LV. Total number of cases with heart thrombi = CT. Number of cases dying of heart disease in the autopsy materials = HD. Total number of autopsies = TA.

difference between the three series regards the left atrial thrombi, which have been observed much more frequently in the present author's series than in the materials of the american authors. To explain this difference, the present author will point out the fact, that rheumatic heart disease was registered in 4.4 per cent of all autopsy cases in series B but only in 1.8 per cent of the autopsy cases in the material of GARVIN. The frequent occurrence of left atrial thrombi in cases with rheumatic heart disease will be commented on later.

The figures for the general frequency of thrombi in the atria and the ventricles may be said to be of roughly the same magnitude. The conditions for the formation of thrombi in the atria and in the ventricles are however generally imagined to be rather different.

The blood stream passing through the *ventricular* cavities is so rapid and powerful that there seems *a priori* to be little reason for reckoning with stagnation factors as important for the formation of ventricular thrombi.

In the *atria* the conditions are somewhat different. Considerable parts of the atrial lumen are apparently placed at the side of the main stream of blood. Especially in dilated atria the auricular appendages may seem to represent backwaters with possibly stagnant contents.

It may be that autopsy specimens are prone to provoke an exaggerated idea of the degree of stagnation within the auricular appendages. Atrial systole will probably effect a rather vivid exchange of blood during life. But many cases had during long periods before death



*auricular fibrillation*, in practice identifiable to a paralysis of the atrial walls.

One may thus assume, that stagnation factors are of greater importance for the formation of thrombi in the atria than in the ventricles. It must however not be forgotten that this conception of stagnation is a relative one, derived from a comparison between the atria and the ventricles. The activity of the ventricles will certainly provide for a rather vivid exchange of blood also in fibrillating atria. The stagnation theory of thrombogenesis was originally applied to the formation of thrombi in the peripheral veins. If the atria were compared with the peripheral veins it should probably seem out of place to speak of the atria as predilection sites of stagnation thrombi.

It is at any rate a widely spread opinion that atrial thrombi might be mainly stagnation thrombi. "Auricular thrombi" have even been referred to as typical examples of this mode of thrombogenesis (ROBERTSON and other authors).

\*

It can be regarded as generally accepted, that *most ventricular thrombi are caused by gross wall lesions, usually ventricular infarcts*. The close relation between ventricular infarction and ventricular mural thrombosis is immediately evident from table 2, showing that in series B ventricular infarcts had actually been registered in 103 out of 193 cases of ventricular thrombosis (53.4 per cent). Coronary heart disease, with or without registered infarcts (the diagnosis group "coronary sclerosis-hypertensive heart disease" — see p. 25) was the main diagnosis in 73.7 per cent of the cases with ventricular thrombi. The predominance of this etiological factor is directly reflected in the *distribution* of the thrombi between the left and the right ventricle (table 1) which is roughly parallel to the one of ventricular infarcts.

The remaining cases form a heterogenous group with respect to diagnosis and in most of them little can be said about the causes for the formation of ventricular thrombi. It is worth mentioning that *only 3 cases with ventricular thrombi had uncomplicated rheumatic heart disease*. The presence of rheumatic heart disease was registered in another 11 cases. Of these cases 9 had extensive ventricular infarcts and the 2 other cases had severe coronary sclerosis but no

Table 2. Distribution of the cases of series B. Site of thrombi and main diagnosis.

		VMI	Cscl + Hpt	Hpt	R	O
Cases with ventricular thrombi	R	0	3	2	1	15
	R+L	25	5	2	—	3
	L	78	31	6	2	15
	Total	103	39	10	3	33
Cases with atrial thrombi	R	20	34	9	17	32
	R+L	3	11	4	18	5
	L	16	14	6	38	22
	Total	39	59	19	73	59
All cases dying of heart disease		181	274	57	151	219

R = right, R+L = right and left, L = left.

VMI = cases with recent myocardial (ventricular) infarcts

Cscl+Hpt = cases belonging to the diagnostic group "coronary sclerosis — hypertensive heart disease" (see p. 25), without recent infarcts.

Hpt = cases with only hypertensive heart disease.

R = cases with rheumatic heart disease. Cases which had in addition myocardial infarcts have been placed in group VMI (ten cases with mural heart thrombi). Cases with bacterial valvular endocarditis have been placed in group O.

O = cases with other heart disease or difficult to place in one of the other groups. Nine cases with mural heart thrombi, in which signs of heart disease had not been registered have not been included in this table.

registered infarcts. Finally there were 5 cases with a bacterial valvular endocarditis and ventricular mural thrombi, more or less directly continuous with the valvular vegetations. They have been included in the group "other heart diseases".

Lesions in the walls of the *atria*, comparable with the ones causing ventricular thrombi, are on the whole little known. To the author's knowledge, the only study which has hitherto been devoted to the question of wall lesions as a cause of atrial thrombi is that of GRAEF, BERGER, BUNIM & DE LA CHAPELLE. These authors studied cases with rheumatic heart disease and they found signs of rheumatic activity in the atrial wall beneath 10 out of 24 thrombi in the *left* atrium.

It is evident from table 2, that cases with rheumatic heart disease (forming a negligible group among the cases with ventricular thrombi) constitute a large diagnosis group among the cases with

atrial thrombi. It is an old experience that cases of mitral stenosis are often complicated with atrial thrombosis, particularly in the dilated left atrium. This fact has been used as an argument for the assumption that atrial thrombi are stagnation thrombi. The observations of GRAEF et al. illustrate the complex character of this question.

Coronary heart disease form however the largest diagnosis group also among the cases with atrial thrombi (in series B). If the cases with rheumatic heart disease are eliminated from this series a very marked preponderance of *right* atrial thrombi becomes evident. It has been mentioned (chapter II) that infarcts are more common in the right than in the left atrium. With the analogous incidence in the ventricles in view it is tempting to conclude, that myocardial infarction may be the chief cause of atrial thrombi too, at least in non-rheumatic cases.

\*

The author concludes, that stagnation factors may be important among the causes for atrial thrombosis. Their importance should however not be overestimated. There is reason to believe that wall lesions, similar to the ones causing ventricular thrombi, are decisive also for the formation of atrial thrombi.

Direct morphological observations in the present author's series A will furnish the facts necessary for a further discussion of the matter.

#### **b. Structural types of atrial mural thrombi. Frequency and types of wall lesion found beneath them.**

In current textbooks little interest is devoted to the structure of mural heart thrombi. The most detailed account known to the present author, is the one given by RIBBERT in the manual of HENKE-LUBARSCH (1924). Briefly, the structure of recent heart thrombi does not differ from the one usually found in "primary" thrombi of other sites. They consist of a skeleton of thrombocytic lamellae with red (erythrocytic) thrombus mass between them. Varying proportions between these two main constituents account for some of the differences in the gross appearance of the thrombi.

The present author has successively learnt to distinguish some

general development types of atrial thrombi, which reflect in a characteristic way the different formation conditions for thrombi in different parts of the atria. These types are no absolute entities, transitional types occur and are common, but the main types are still commoner and easy to recognize. The author will base the following survey on a description of these types.

The main types of wall lesion found beneath the thrombi will in this chapter simply be registered as occurring or not occurring. They will be described in detail in chapters VI and VII. Here it must however be mentioned that only reasonably extensive and well-defined lesions have been accepted as possible causes for the formation of mural thrombi. No attention has been paid to diffuse or minor lesions (endocardial thickening or scarring, scattered myocardial round cell foci or perivascular scarring in the myocardium, diffuse interstitial fibroses, to mention some examples) which may be observed in most cases dying of heart disease. Whether the lesion could be accepted as important or not had to be decided in a rather arbitrary way. Actually the decision was, as a rule, not very difficult to make.

It is most convenient to begin with a description of the types of thrombi occurring in the

#### LEFT ATRIUM.

1. The term recess thrombi will in this study be used for a characteristic type of thrombi occurring in the trabeculated part of the left atrium. Such thrombi are isolated, more or less smoothly rounded formations, lodging within the marginal or apical caverns of the auricular appendage. They are sometimes apparently not at all fixed to the wall, but too big to pass out through the narrow opening of the cavern, otherwise the attachment surface is usually small, sometimes represented by a tiny stalk. Consequently recess thrombi are easily lost or dislodged when the left auricular appendage is opened in autopsy.

Because of the defective contact with the heart wall the organization of recess thrombi has often to begin in a very restricted spot and the author has often seen histological pictures, suggesting that the organization of recess thrombi may be a very slow process. The ultimate result may be that of stalked fibrous "polypi", but the author must state, that most completely organized thrombi observed

in the left auricular appendage were attached rather broadly to the wall.

Most recent recess thrombi seem to have little to do with the walls of the recess holes in which they lodge, and their general appearance cannot be said to suggest wall lesions as important causal factors.

As a matter of fact significant wall lesions were seldom found in the atrial walls adjacent to recess thrombi. Thrombi within the left auricular appendage were present in 71 cases of series A. More or less extensive infarcts (or "minor lesions", see p. 70) were present in 3 of them and a mural endocarditis in another 3 cases. There was one case with a diffuse vacuolar degeneration of the myocardium of uncertain etiology. In the remaining cases no or inconspicuous wall lesions were found.

It may be mentioned that rather varying diagnoses were represented among the cases with recess thrombi. Rheumatic heart disease was the main diagnosis in 29 cases and 26 cases belonged to the coronary sclerosis-hypertension diagnostic group, 13 of which had acute ventricular infarcts. Miscellaneous diagnoses or coinciding rheumatic and coronary heart disease were registered in the remaining cases.

2. The term surface thrombi of the left atrium will be used for a quite different type of thrombi.

Surface thrombi are usually large, flat formations, broadly and firmly attached to the endocardium in the *smoothwalled part* of the atrium. At any rate, they attain their typical appearance only in this part of the atrium.

The author has never seen a quite recent thrombus in this part of the heart. It must be assumed, that the development of surface thrombi usually begins at least several weeks before death. Some of them are probably very old formations, representing a *chronic* type of heart thrombosis. They have usually a rather uniform general structure corresponding more or less completely to the following description. (See figs. 10, 11, 12.)

A thick layer of dense, fibrous tissue is regularly found close to the endocardium. It probably represents the completely organized bases of an old thrombus. It may be mistaken for a sclerosed endocardium, but as a rule the original endocardium may be distinguished from the fibrous bases of the thrombus by means of its inner elastic

membrane and by the fact that it always contains smooth muscle cells.

The *marginal* regions of surface thrombi are as a rule completely organized; no remnants of the original thrombus substance are left and the lumen surface may be quite smooth and similar to normal endocardium.

Instead the *central* parts of such thrombi are usually incompletely organized, and the fibrous basis is most often covered by hyaline remnants of the original thrombus substance. Such hyaline thrombus substance may be present in several layers, probably of different age. It should directly face the lumen — as a central “ulcer” of the surface thrombus — were it not regularly covered by recent vegetations of mainly thrombocytic thrombi. There is usually a very distinct demarcation between old and recent thrombus substance at this level. Such recent vegetations have obviously little chance of getting organized from the acellular surface, on which they develop, consequently they do not become firmly adherent and are easily dislodged.

There is an obvious similarity between the structure of surface thrombi and the structure of callons (peptic or varicose) ulcers. The author has consequently called them too *callous thrombi*. Probably the similarity is not only a superficial one, the same reasons may in both instances account for the defective healing tendency in the center of a large, fibrous scar.

The description given may seem to correspond only to *one stage* in the development of surface thrombi. It must at any rate be a stage of long duration. The author has seldom seen completely organized surface thrombi, central “ulcers” will probably for long periods withstand the organization process representing important sources for systemic embolias.

Surface thrombi are not as common as recess thrombi, but they are not at all rare. Of 98 left atrial thrombi in series A, 31 belonged more or less distinctly to this type. Of these thrombi 25 were found in cases with mitral stenosis. Coronary sclerosis or hypertensive heart disease were registered as main diagnoses in the remaining cases.

It is difficult to explain the presence of these thrombi on the smooth wall of the main atrial lumen, without assuming wall lesions as a main cause. As a matter of fact a rather uniform type of round-cell endocarditis (to be described in chapter VI) was present under the

thrombi in 22 of these 31 cases. In the remaining cases conspicuous scarring and in 2 cases even calcification of the endocardium was observed, but no cellular infiltration.

### RIGHT ATRIUM.

The types of thrombi to be found in the right atrium differ in some respects from the ones found in the left atrium.

The author has never seen mural thrombi in the smooth-walled part of the right atrium. They were all found in the trabeculated part, which in the right atrium includes, not only the auricular appendage, but also considerable part of the wall surface of the main atrial lumen. Consequently typical surface thrombi were not found in the right atrium.

Most thrombi were found within the numerous sinuositities which are typical of the right atrium. The complicated branchery of trabeculae filling up the auricular appendage and the auricular sinus prevent such thrombi from attaining the "corpus liber" type of left recess thrombi. They may instead attain considerable dimensions within the extensive recess "systems" of the auricular sinus, which they often fill throughout. Very often however, numerous small, rounded clots are seen to protrude between the pectinate muscles. They are usually arranged in groups, covering limited areas of the atrial wall, and the author believed originally, that they indicated lesions in this very segment of the wall. As a matter of fact they proved usually to be but sprouts from a continuous thrombus mass filling an auricular sinus. Such groups of small thrombi represent typical findings in the right atrium; because of their similarity with mushrooms, sprouting between the roots of a tree the author has called them fungiform thrombi.

Recent fungiform thrombi are as a rule rich in erythrocytes and their inner parts have a darkly red colour. They are however usually covered by a thin layer of fibrine, making them appear white or bluish from without. In sections this "limiting membrane" is seen to separate also the edges of the thrombocytic lamellæ (lines of ZAHN) from the atrial lumen, apparently marking that the growth of the thrombi is finished. All recent fungiform thrombi in the same group represent the same stage of development and pictures suggesting a stage of actual increase are seldom seen. The author consequently

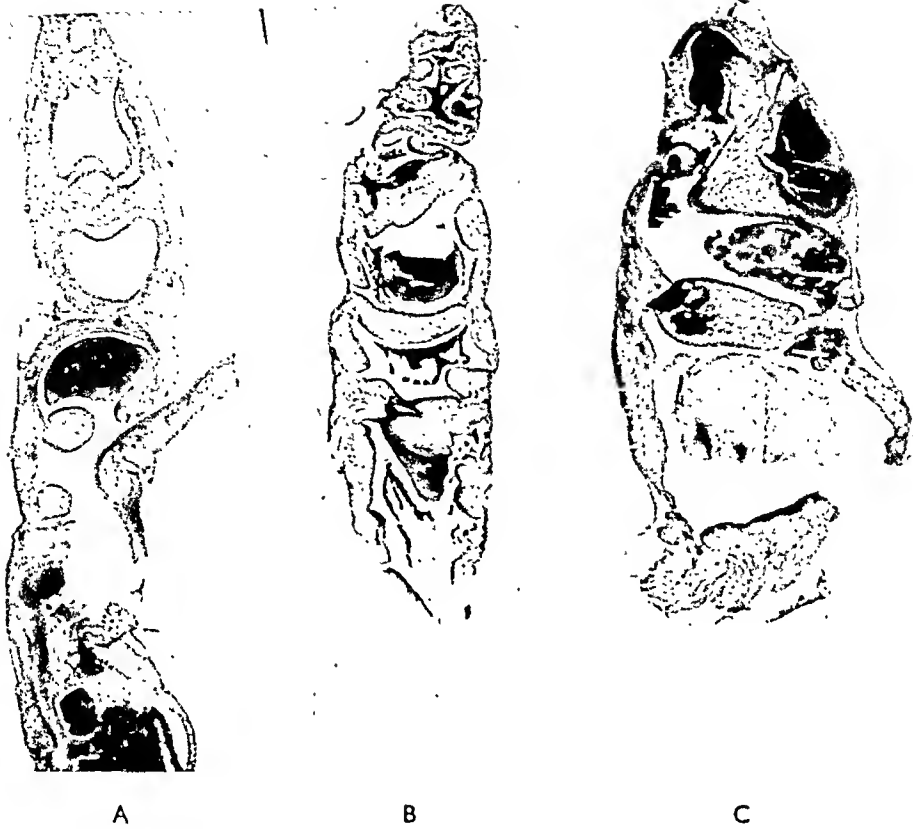


Fig. 9. Left auricular appendages with recess thrombi (Ser. A, cases 140 (A) 121 (B) and 19 (C), v. Gieson,  $\times 3$ ).



Fig. 10. Typical surface thrombi of the left atrium. (Ser. A cases 37 (A), 99 (B) and 10 (C), v. Gieson,  $\times 2 \frac{1}{2}$ ).



A



Fig. 11. Surface thrombus. Mural endocarditis.  
I myocardium, II endocardium, III fibrous basis of the thrombus, IV old thrombus substance, incompletely organized. (ser. A, case 7, v. Gleson, A  $\times 30$ , B  $\times 75$ ).

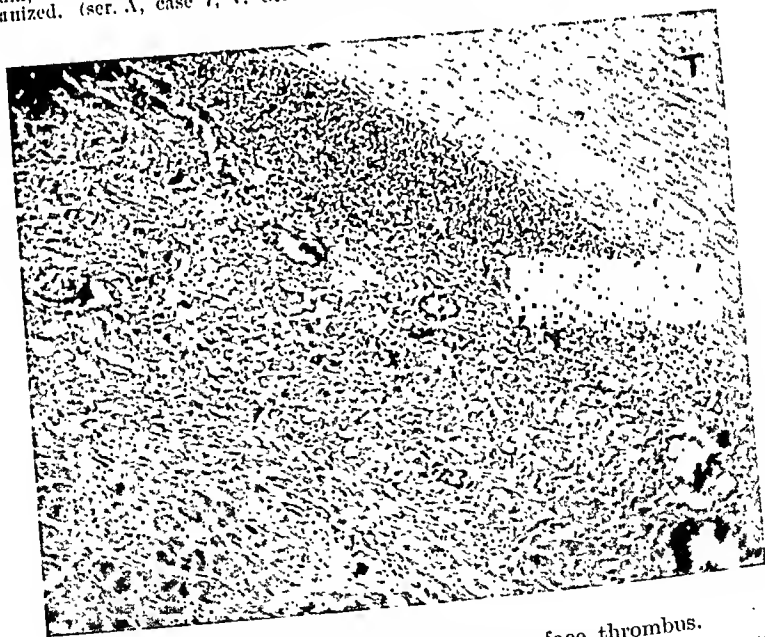


Fig. 12. Endocardial round cell focus beneath a surface thrombus.  
T fibrous basis of the thrombus. Indistinct demarcation between the myocardium and the endocardium. (Ser. A case 57, v. Gleson,  $\times 75$ ).

A



B

Fig. 13. Mural endocarditis. Detail of a round cell focus. (Ser. A case 85 v. Gieson,  $\times 210$ ).



Fig. 14. A. Recent fungiform thrombi in the right atrium. Puriform softening of the thrombus center. (Ser. A case 46, v. Gieson,  $\times 3$ ). B. Tangential thrombus of the right atrium. The thrombus is completely organized. The light spots in the trabeculae represent the scarred "central zones" of an old infarct. (Ser. A case 112, Hematoxylin — Sudan III,  $\times 3$ .)



Fig. 15. The wall of a thrombus cyst.  
 A = lumen surface. The thin limiting membrane is still not organized. B = inner surface of the thrombus cyst. (Ser. A, case 46, Weigert's fibrin stain,  $\times 48$ ).



Fig. 16. Thrombus cyst, somewhat older than the one in fig. 15.  
 The limiting membrane (A) is organized and distinctly demarcated from the rest of the thrombus. B is the liquefied center of the thrombus.

(ser. A, case 122 v. Gleason,  $\times 48$ ).

assumes, that the formation of fungiform thrombi is accomplished within a short time and simultaneously stopped in all the individual sprouts.

It is evident, that the organizational conditions must be nearly ideal for the parts of the thrombi enclosed in the auricular sinus because of the extensive surface of contact between thrombus and endocardium. The final result will simply be an obliteration of the intertrabecular spaces with fibrous scar tissue.

Conditions are different for the protruding globuli forming the fungiform thrombi. The organization is, during a first stage, apparently restricted to the surface of such thrombi and it may sometimes be seen that the fibroblasts actually follow the "limiting membrane" mentioned above. In early stages fungiform thrombi become thus enclosed within a thin capsule of new connective tissue (fig. 16). The fibroblasts seem on the other hand to have little tendency to migrate towards the inner parts of the thrombi. Fungiform thrombi are instead often liquefied in their central parts, a process called *puriform softening*.

It is the present author's impression that most liquefied thrombi burst (at least the larger ones), emptying their contents into the atrial lumen. A stage often met with in autopsies is at least that of empty thrombus cysts, similar to burst puffballs, with the flaccid cyst walls projecting into the atrial lumen from the fibrous basis of the thrombus. The gross appearance is however often confusing, the finding may be mistaken for representing quite recent thrombi consisting of loose, irregular, ragged clots in a first stage of increase. Microscopic control is often necessary to reveal the real state of affairs.

Liquefaction eliminates most of the protruding parts of the right atrial thrombi. Between the numerous trabeculae of the right atrium only short distances then remain to be bridged over by the organization process. As a consequence, the lumen surface of old thrombi in the right atrium is usually completely organized and "central ulcers" are seldom seen. Such remnants of thrombi are often difficult to recognize with the naked eye, but the author has sometimes registered them as quite unexpected findings in slides from the right atrium.

The general structure of right atrial thrombi does seldom permit direct conclusions as to the etiology. In a few cases with atrial in-

farcts, the author did however observe a type of thrombi, which in this respect were comparable to left atrial *surface* thrombi. The author has called such specimens *tangential* thrombi (fig. 14 B). They had the appearance of flat discs fixed to the crests of the pectinate muscles and bridging over the grooves between them. The formation of such thrombi may be explained by the fact that the myocardial necrosis becomes manifest only in the more massive pectinate muscles, the remaining part of the atrial wall being sheltered by the lumen zone and the pericardial zone (chapter VII).

Among the 138 cases with right atrial thrombi (series A) 23 had only rheumatic valvular disease and 76 belonged definitely to the diagnostic group of coronary-sclerosis — hypertensive heart disease. Acute ventricular infarcts were present in 30 cases in the latter group.

Significant wall lesions were found under the right atrial thrombi in 87 (63.5 per cent) of the cases. Lesions of a "coronary" type were observed in 79 cases (46 cases with "atrial infarction" and 33 cases with "minor lesions" according to the definitions to be found in chapter VIII). Of the remaining cases 2 had myocardial amyloidosis; a single case of mitral stenosis had an acute endomyocarditis under a thrombus in the right atrium. The lesions in the remaining 6 cases were labelled as myocarditis or myocardial degeneration of uncertain etiology.

It must be added, that the figures given for the occurrence of "coronary" lesions beneath right atrial thrombi are probably too low. During earlier periods of the investigation, blocks were sometimes taken only from the center of the thrombus-covered part of the atrial wall. It is evident from the preceding discussion that this in most cases meant *from the mid-part of the corresponding auricular sinus*. It was only successively learnt by the author that the infarct area often but touches the extremity of such a sinus, the thrombus induced will nevertheless fill the sinus throughout. Such a discrepancy between the extension of infarct and mural thrombus is evident in fig.

\*

Very substantial and typical wall lesions were thus registered under the atrial thrombi in about half the cases. In the *right* atrium more or less extensive "coronary" myocardial lesions (infarcts and minor

lesions; definitions see chapter VIII) were the typical findings, present under about  $2/3$  of the thrombi. Considering the rarity of atrial infarcts in cases without thrombi (see chapter VIII) it may seem justified to assume, that *most right atrial thrombi are caused by myocardial infarcts*. In the *left* atrium myocardial infarcts were exceptional. A lesion characteristic of the left atrium was instead that of *mural endocarditis* occurring beneath a type of thrombi (surface thrombi) the very position of which indicated that the lesion of the atrial wall must have been the decisive cause for their formation. Mural endocarditis was however found only beneath about  $1/4$  of all left atrial thrombi.

The author can obviously not pretend to have proved the absence of causal wall lesions under the thrombi in all the remaining cases. However it is probably significant, that wall lesions were exceptional findings in the large and rather well defined group of left "recess thrombi". It is possible that some of these thrombi have been caused by *minute lesions*, which may happen to fall without the isolated sections available. One may or may not assume the presence of such wall lesions, it is at any rate possible that *stagnation factors* had been of decisive importance for the formation of these thrombi.

### c. Relations between auricular fibrillation and atrial mural thrombi.

A closer analysis of the importance of *stagnation factors* (as represented by congestive failure, atrial dilatation or auricular fibrillation etc.) for the formation of atrial mural thrombi is a very difficult enterprise. They often more or less coincide and it is difficult to get materials for comparison in order to check the importance of the different factors. The author will in this chapter restrict himself to a study of the importance of *auricular fibrillation*, a condition which is at least not very difficult to define.

It is mentioned in many textbooks as a matter of fact, that atrial thrombi are more frequent in cases with auricular fibrillation than in other cases. The causal relations may seem to be evident; it is hardly more than a truism to state that a suspended systolic evacuation of the atria must mean a favourable condition at least for the growth of mural thrombi. In order to illustrate another effect of auricular fibrillation, it may be said that the immobility of the

Table 3. Incidence of auricular fibrillation in series B.

	All heart thrombi			O	Atrial thrombi		
	A	A+V	V		R	R+L	L
T.....	192	58	126	497	115	41	90
A F.....	124	18	20	184	47	30	65
% A F of T	64.6	31.0	15.9	37.0	40.9	73.2	70.0
	$\pm 3.45$	$\pm 6.07$	$\pm 3.26$	$\pm 2.2$	$\pm 4.59$	$\pm 6.92$	$\pm 4.73$

T = total number of cases. A F = cases with auricular fibrillation

A = cases with atrial thrombi only

A + V = cases with atrial and ventricular thrombi

V = cases with ventricular thrombi only

R = cases with right atrial thrombi only

R + L = cases with right and left atrial thrombi

L = cases with left atrial thrombi only

O = cases without heart thrombi with heart disease as a main cause of death.

auricular walls makes them as defenceless against thrombotic deposits, as a horse should be against flies without his cutaneous muscles.

It must however be stated that statistical proofs for the connection between auricular fibrillation and atrial thrombi have not often been furnished in the literature. The question has been studied by HARVEY & LEVINE (1930) who in a total material of 111 heart thrombi found *atrial* thrombi in 88 per cent of the fibrillating cases, but only in 54 per cent of the cases with regular rhythm. They concluded that auricular fibrillation was important as a cause of atrial thrombi. In a later study HAY and LEVINE (1942) registered the frequency of atrial thrombi in 186 cases of mitral stenosis and noted a significantly higher incidence of mural thrombi in cases with auricular fibrillation than in cases with regular sinus rhythm. WEISS and DAVIS (1933) published an analysis of 154 autopsy cases of rheumatic heart disease and noted, that in 22 out of 25 cases with *extensive* atrial thrombosis (88 per cent) fibrillation had been present during life. They concluded that fibrillation was an important cause of atrial thrombi. Finally GARVIN found only in cases with rheumatic heart disease a higher incidence of heart thrombi in cases with fibrillation than in cases with regular rhythm. GARVIN made in this statement no difference between atrial and ventricular thrombi.

The incidence of auricular fibrillation in the *present author's series B* is considerably higher among the cases with *atrial* thrombi than

Table 4. Incidence of auricular fibrillation in series B. Cases of *rheumatic heart disease* with *atrial* thrombi. The figures comprise all cases with rheumatic valvular disease.

	NT	R	R+L	L
T.....	137	22	19	41
A F.....	52	11	13	33
% A F of T.....	38.8	50.0	68.4	80.5
	$\pm 4.3$	$\pm 10.7$	$\pm 10.7$	$\pm 6.2$

NT = cases registered as dying of heart disease in which signs of rheumatic valvular disease were found but no heart thrombi.

Other abbreviations, see table 3.

among the cases without mural heart thrombi, dying of heart disease (Table 3).

According to the same table the incidence of auricular fibrillation was instead significantly *lower* among the cases with *ventricular* thrombi than among the cases without heart thrombi. This observation may seem surprising. It is hardly probable, that the auricular fibrillation in itself exerts any influence on the formation of ventricular thrombi, at least not that it *inhibits* their formation. There is, however, a probable and rather instructive explanation to this fact: most ventricular thrombi are found in cases with ventricular infarcts, and auricular fibrillation is not common in cases with ventricular infarcts. The same mode of reasoning may be applied also to the question of atrial thrombosis: it is by no means certain, that a positive correlation between auricular fibrillation and atrial thrombi is due to fibrillation as a *stagnation* factor. Fibrillation and atrial thrombi could be the parallel consequences to a common causal factor (possibly wall lesion).

Returning to the atrial thrombi, it may be stated from table 3 that there is a very high incidence of auricular fibrillation among the cases with *left* atrial and *bilateral* thrombi. Most *right* atrial thrombi, however, are found in cases with regular sinus rhythm, and the incidence of auricular fibrillation in this group is not significantly higher than in the group of cases without heart thrombi.

It is inviting to assume, that this difference between right and left atrial thrombi is due to the fact that left atrial thrombi are particularly common in cases of rheumatic heart disease. It has been



Table 5. Incidence of auricular fibrillation in series B. Cases of *non-rheumatic* heart disease with *atrial* thrombi. The figures comprise all cases without signs of rheumatic valvular disease.

	NT	R	R+L	L
T .....	360	93	22	53
A F .....	132	36	17	32
% A F of T .....	36.7	38.7	77.3	60.4
	$\pm 2.6$	$\pm 5.1$	$\pm 8.9$	$\pm 6.7$

NT = cases registered as dying of heart disease in which no signs of rheumatic valvular disease and no heart thrombi were found.

Other abbreviations, see table 3.

mentioned that a positive correlation between fibrillation and atrial thrombosis has been demonstrated by previous authors in this very type of heart disease. This assumption is however not confirmed by the figures in tables 4 and 5. Both in cases with rheumatic heart disease and in cases without rheumatic heart disease the typical difference is present between right and left atrial thrombi with respect to their relations to auricular fibrillation.

It is obviously *not* permissible to conclude from these statements that auricular fibrillation is of no consequence for the formation of right atrial thrombi. One may however safely conclude, that most right atrial thrombi are caused by factors which are *not* connected with auricular fibrillation. This conclusion will be of some interest for the discussion (chapter IX) of auricular fibrillation as a possible consequence of atrial infarction.

It may furthermore be concluded, that the formation of atrial thrombi in cases with auricular fibrillation should not simply be regarded as due to the *mechanical* effect of the fibrillation. Auricular fibrillation is always a bilateral condition, and there is no reason to assume that its influence on the formation of thrombi is another in the right than in the left atrium. It is difficult to explain (at least in the cases of rheumatic heart disease) the high incidence of left atrial thrombi in fibrillating cases as due to the mechanical effect of fibrillation if no such effect is demonstrable for the part of the right atrium. It may be assumed, that factors causing left atrial thrombi may be the direct causes also for the appearance of auricular fibrillation.

## Summary.

It was possible to distinguish in the *left atrium* two development types of mural thrombi. *Surface thrombi* develop in the smooth-walled part of the left atrium, they are probably in most cases caused by a mural endocarditis. *Recess thrombi* occur in the left auricular appendage, significant lesions are only exceptionally found in the atrial walls under such thrombi. Analogous types of thrombi could usually not be distinguished in the right atrium. *Right atrial* thrombi are *rather uniform* and develop in remote recesses; they may reach considerable extension within the auricular appendage and the auricular sinuses. Wall lesions, usually more or less extensive myocardial infarcts, were found beneath most right atrial thrombi.

There is reason not to exaggerate the importance of stagnation factors for the formation of atrial thrombi. The relations of *auricular fibrillation* to atrial thrombosis was studied in a large material. It turned out, that cases with *left* atrial thrombi showed a higher incidence of auricular fibrillation than other groups of cases dying of heart disease. No significant correlation could be demonstrated between *right* atrial thrombi and auricular fibrillation.

## CHAPTER VI.

### Mural endocarditis of the left atrium.

It was pointed out in chapter V, that *mural endocarditis* is probably an important cause for the formation of left atrial "surface" thrombi. Little attention is usually paid in routine autopsies to this type of endocarditis and the author feels justified in recording some observations regarding this typical lesion of the atrial wall.

The previous literature on this topic has been reviewed in a peremptory way by Gross (1935). It has long been known, that an involvement of the mural endocardium of the left atrium is common in cases of subacute bacterial endocarditis. The first author to point out the common occurrence of such an endocarditis in cases of uncomplicated rheumatic heart disease was MACCALLUM (1924) ("MACCALLUM patch"). v. GLAHN (1926) observed in 9 out of 31 cases of rheumatic heart disease a mural endocarditis with a patchy distri-

bution over the inner surface of all the left atrium. GROSS (1935) found in a material of 87 cases of rheumatic heart disease some sign of mural endocarditis in all cases. He regards however mural endocarditis to be present also in cases, in which the alterations consisted mainly of scarring and vascularization of the endocardium without conspicuous cellular infiltration.

According to these authors the inflammatory cells consisted mainly of lymphocytes but also, in the acute stages, of polymorphonuclear leucocytes, MACCALLUM found, in addition, typical ASCHOFF bodies. v. GLAHN found no typical ASCHOFF bodies but large, basophilic cells arranged to form "palisades" parallel to the endocardial surface. v. GLAHN and GROSS regarded however all the types of cells characteristic of the ASCHOFF bodies to be present in some cases of mural endocarditis, they assumed that only the anatomical structure of the endocardium prevents the development of distinct ASCHOFF bodies of the habitual type.

The authors mentioned did not observe the formation of mural thrombi as a consequence of mural endocarditis. The presence of a causal relation between mural endocarditis and mural thrombosis has hitherto been suggested only by GRAEF, BERGER, BUXIM & DE LA CHAPELLE (1937). They found in cases of rheumatic heart disease a mural endocarditis beneath 10 out of 24 left atrial thrombi, apparently both in the trabeculated and in the smoothwalled part of the atrium. They concluded that mural endocarditis was an important cause for the formation of left atrial thrombi.

In the present author's material the diagnosis of a significant mural endocarditis was based on the presence of a fairly uniform histological picture. The main feature was an infiltration of the endocardium with round cells (lymphocytes and plasma cells). Larger cells, comparable to the large cells occurring in ASCHOFF bodies were seldom observed. As a rule the cells were arranged in large, irregular foci *near to the myocardium*, sometimes there was a more or less continuous infiltration throughout the endocardium, which in 2 cases was filled with tightly packed round cells (fig. s. 11, 12 and 13). Sometimes the continuous cellular infiltration extended also to adjacent parts of the myocardium. Very often a degeneration of muscle fibres and an increase of the interstitial connective tissue could be observed in the layer of myocardium immediately outside the endocardium (fig. 12). As a consequence, the usually sharply

defined limit between the myocardium and the endocardium was in such cases often indistinct.

It must be emphasized, that this type of endocarditis may easily be distinguished from the leucocytic or in later stages lymphocytic cellular infiltration, which often accompanies the organization of mural thrombi. This type of cellular infiltration is regularly most pronounced in the thrombus itself, but may extend also to the inner (subendothelial) layer of the endocardium, which may consequently become considerably scarred. Scarring and vascularization of the endocardium is a regular consequence of the organization of mural thrombi, and its presence does not indicate, that an endocarditis preceded the formation of thrombi.

In this study mural endocarditis was diagnosed only in cases with a conspicuous *cellular infiltration* in the outer layers of the endocardium (called by GROSS the subendocardium).

Typical endocardial or subendocardial ASCHOFF bodies were not observed in the author's material (not even the "banded ASCHOFF bodies" described by v. GLAHN and GROSS). Rheumatic heart disease was however present in 22 of the 25 cases registered and it is consequently very probable that this type of mural endocarditis is a manifestation of rheumatic heart disease. The non-rheumatic character of the remaining cases (2 cases labelled as coronary sclerosis and one as hypertensive heart disease) can not be regarded as established with certainty.

The author has found no clinical signs to be referred specially to the left atrial mural endocarditis. Subacute bacterial valvular endocarditis was present in only one of the cases.

The incidence of auricular fibrillation was about the same in the cases with mural endocarditis as in the other cases with left atrial thrombi.

## CHAPTER VII.

### The microscopical picture of atrial infarction.

It was pointed out by CUSHING et al., that atrial infarcts are easily overlooked in routine autopsies. The present author maintains that without microscopical examination they can only be diagnosed

with reasonable accuracy in exceptional cases. But the histological diagnosis also entails some little known difficulties and the findings differ in many respects from the customary picture in ventricular infarcts. A study of the factors constituting the difference proved to yield some facts of general interest for the understanding of myocardial lesions in coronary disease. The author will consequently discuss the histological findings in some detail.

#### **a. General facts regarding the histology of myocardial infarcts.**

Literature as to the microscopical appearance of myocardial (ventricular) infarcts is rather scanty. Most authors refer to the papers of KARSNER & DWYER, studying experimental (ventricular) infarcts in dogs, and of MALLORY, WHITE & SALCEDO-SALGAR, describing the histology of human (ventricular) infarcts in different stages of development. Their accounts agree quite well; the only important difference is, that the stages of repair follow one another more quickly in the dog than in man. The following survey refers mainly to the description of MALLORY et al.

According to these authors the first histological signs of myocardial infarction can be observed 5--6 hours after stoppage of the arterial blood supply. The most striking sign is, that the affected muscle fibres appear hyaline and take a deeper acid stain than normal fibres. During the following hours this change becomes more marked. The cross-striation of the fibres in such hyaline necrosis is usually difficult to discern but does not always disappear — the author has often seen it rather exaggerated in fully developed hyaline necrosis.

The behaviour of the nuclei of the muscle cells is not commented on by MALLORY et al.; they are known to disappear as quickly in necrotic myocardium as in other necrotic tissue. The present author must however stress that they may be plainly visible even when the picture of hyaline necrosis is quite distinct.

Infiltration of the necrotic tissue with polymorphonuclear leucocytes begins after 24 hours, and during the following week rows of leucocytes between the myocardial fibres form a prominent and typical feature of the picture. Organization, with ingrowth of capillaries and fibroblasts and removal of necrotic muscle, begins about the

fourth day; in an infarct of 10 days standing MALLORY et al. found the breadth of the organization zone to be 1 mm. The time necessary for the removal of the necrotic remnants varies considerably with the extension of the infarct but the development of the scar tissue is comparatively quickly accomplished, the formation of collagen fibrils reaching its maximum within 2—3 months. MALLORY et al. found a fibrinous pericarditis after 24 hours in many cases, but mural thrombosis did not appear before the fifth day.<sup>1</sup> They note, that recent mural thrombi could also be found in the late stages of repair of myocardial infarcts. Finally they observed along the endocardium a narrow zone of surviving myocardium, which must have been nourished directly from the heart lumen. This zone will be called in this study the lumen zone. A lumen zone has been observed by several previous authors, but as a rule little attention has been paid to it. Its presence is however of decisive importance for the development of the peculiar histological picture of atrial infarction.

According to this survey, the development of myocardial infarction may in histological terms be expressed simply as the *appearance of a complete hyaline necrosis, its successive removal and its final replacement by scar tissue.*

This is however no complete description. Some other types of myocardial degeneration have also been observed and recorded in the literature concerning myocardial infarction.

MALLORY et al. state that considerable amounts of fat (or lipoids) are present within the myocardial fibres only if the myocardium has been insufficiently nourished *before* the onset of a sudden arterial occlusion. Otherwise they found fatty degeneration of the fibres only in the periphery of the infarcts. KARSNER & DWYER report similar observations.

There is another type of myocardial degeneration in infarct cases which has been cursorily referred to by some authors as "vacuolar degeneration". A good microphoto, representing this type of degeneration, is reproduced by BOYD in his book "Pathology of Internal Diseases" (p. 66). This type of myocardial alteration is often a dominating finding in atrial infarcts and will become a main object of study in this chapter.

It is the present author's experience, that distinct vacuoles are

<sup>1</sup> BEAN noted in some cases mural thrombosis 24 hours after the onset of infarction.

usually not present in the fibres presenting this type of degeneration. The fibres appear rather diffusely inflated and poor in fibrils. The present author found often fibres having this appearance in the transition zone between hyaline necrosis and normal myocardium. Under the microscope such a zone is often seen to surround the areas of hyaline necrosis as a rim of foam. In the present study the term *foamy degeneration* will be used for this type of degeneration. It probably represents a degree of damage next to complete necrosis.

The present author should like to briefly define the histological picture of recent (ventricular) myocardial infarction in the following way:

A large *central* zone of hyaline (complete) necrosis is surrounded by a narrow border of degenerated tissue which is not definitely necrotic and which usually presents the histological picture of *foamy degeneration*. The most conspicuous part of this border zone is the *lumen zone*, separating the zone of hyaline necrosis from the endocardial surface.

#### **b. The special histological picture of atrial infarcts.**

The histological findings in atrial infarcts do not deviate in principle from the account given above. In most cases, however, the actual picture seems to differ considerably from the one usually seen in ventricular infarcts.

The reason is easy to understand considering two of the statements of MALLORY et al. They state that the zone of intact myocardium beneath the endocardium has a breadth of 0.3—0.5 mm (the figures of the present author are somewhat lower — see p. 62). If such a lumen zone is assumed to be present in infarcts of the thin atrial wall, there will be but little room for complete hyaline necrosis in most atrial structures. Furthermore, if all traces of necrotic muscle may be removed within 10 days to a depth of 1 mm from the margins of the infarct, then it must only be possible to find the typical hyaline necrosis in the atrial walls during a very short space of time after the occlusion of an artery.

It is easy to imagine that in the *left atrium*, the blood content of which is well oxygenated, the conditions mentioned may prevent the development of a plain histological picture of myocardial infarction.

The position is different in the *right atrium*. Its blood content is venous; in congestive failure the oxygen saturation certainly often falls below 50 per cent. It may be assumed that the nutrition of the lumen zone after infarction of the right heart will be rather defective. The actual findings agree well with this assumption. The lumen zone of right atrial infarcts present regularly more or less conspicuous signs of degeneration, most often foamy degeneration.

Manifest hyaline necrosis is, however, little prominent in sections from *right atrial* infarcts too and it is, at the first glance, often difficult to reveal the true nature of the lesion also under the microscope. Most of the myocardium belongs to the lumen zone (or to a corresponding but less constant pericardial zone — see p. 63) and presents usually some sign of degeneration but no necrosis. The scattered areas of hyaline necrosis present in recent infarcts may be very inconspicuous (fig. 18 A) and in later stages the patchy scarring in the central parts of the trabeculae is also easily overlooked. In large areas there is nothing more to be found than a diffuse foamy degeneration, a diffuse, more or less pronounced infiltration with leucocytes or sometimes round cells and finally a more or less diffuse interstitial fibrosis. Such findings are easily labelled simply as a diffuse myocarditis. Recent infarcts in particular may be very difficult to recognize.

The cause of this confusing picture is obviously the lumen zone, protecting large parts of the atrial walls from the extreme results of a deficient coronary blood supply. *But the demonstration in suitable places, of a distinct lumen zone is also a crucial point for diagnosis.* In this investigation the demonstration of a lumen zone has been used as an absolute condition for the diagnosis of "coronary" lesions in the myocardium.

Lumen zone, pericardial zone and foamy degeneration constitute together the typical picture of atrial infarcts and will form the subjects of special descriptions.

### c. Lumen zone.

Several authors studying the histology of atrial infarcts have observed the existence of a lumen zone (Cfr MALLORY et al.). Its value as a criterion of »coronary» lesions has not been pointed out previously.



In atrial infarcts it is usually to be seen in all structures sufficiently massive to permit a clear differentiation into central zone and lumen zone. It penetrates into the central zone along the thin-walled vasa minima (figs. 20, 21) and it may also be seen around the rather constant central artery of the "pectinate muscles" (fig. 19 — cf the observations of WEARN et al. quoted on p. 32).

The lumen zone is sometimes, but not always absent beneath mural thrombi (figs. 17 A, 18 B). The lumen zone visible under mural thrombi is often as completely necrotic as the central zone but the different age of the necrosis accounts for the different appearance. Within large thrombi small trabeculae may be found to be completely necrotic even in definitely non-"coronary" cases. Possibly trabeculae of this type are *normally* absolutely dependent on nutrition from the lumen.

There is usually a distinct demarcation between the lumen zone and the central zone. As a rule the degree of degeneration is about the same throughout the whole breadth of the lumen zone, sometimes, however, (specially in the left ventricle) some muscle fibres nearest to the lumen seem to be better preserved.

In sections from the same case and the same part of the heart the *breadth* of the lumen zone is usually constant. In different cases the figures may differ considerably. Several factors probably influence the breadth of the lumen zone; specially interesting from the author's point of view is the influence of the oxygen saturation in the lumen blood.

In order to study the importance of this factor, the author measured the breadth of the lumen zone in 29 cases of right atrial infarct and 16 cases of left ventricular infarct. For these measurements the author selected trabeculae cut transversely, the endocardium of which was not more than 0.025 mm thick. The lumen zone was measured from the central zone to the endocardial surface of the *myocardium* (the figures consequently do not include the breadth of the endocardium). The smallest breadth to be measured in such trabeculae was regarded as the real one. The mean of the figures from 2—3 points of measurement was registered in every case; usually the differences between the figures from the same case were very small.

The mean breadth of the right atrial lumen zone in this series was  $0.13 \pm 0.007$  mm and the range of variation from 0.07 to 0.20

mm. Corresponding figures for the lumen zone in the left ventricle were: mean breadth  $0.23 \pm 0.015$  mm and range of variation from 0.07 to 0.35 mm. The difference between the mean breadths ( $0.10 \pm 0.017$  mm) is statistically significant.

There are good reasons to assume that the presence of a lumen zone is due to a nourishment of this part of the myocardium directly from the lumen blood. It is very inviting to conclude, that the broader lumen zone in the left ventricle is due to the higher oxygen saturation in the blood in the lumen of the left heart. Unfortunately left atrial and right ventricular infarcts, which could furnish the final proofs for this theory are of very rare occurrence. In the only case of left atrial infarction observed by the author no suitable places for the measuring could be found. In 3 cases of right ventricular infarction the lumen zone was rather narrow (0.135, 0.08 and 0.075 mm), but the number of cases is too small to permit definite conclusions. The present author finds the assumption probable, that the lumen zone in the left atrium has a breadth corresponding to that found in the left ventricle; this assumption contributes to the explanation of the rarity of left atrial infarcts (and consequently of measurable left atrial lumen zones).

#### d. Pericardial zone.

A zone of surviving myocardium may also be found immediately under the pericardium. It is however not so constantly present as the lumen zone. In addition the demarcation line between this pericardial zone and the central zone is seldom sharply defined and the breadth of the pericardial zone may vary within very wide limits also in the same slide.

It is not easy to give a satisfactory explanation for the presence of a pericardial zone. It has been vindicated by some authors, that the increased pressure in the myocardium during systole should present a significant obstacle for the coronary blood supply to the inner layers of the myocardium (cf. LEPESCHKIN § 770). It is consequently possible, that the layers of myocardium immediately beneath the pericardium are in a favourable position with regard to the coronary blood supply. It is, in addition, not quite impossible that a diffusion of oxygen from vessels ramifying in the parietal pericardium could be of some importance in this connection.

Whatever the explanation may be for the presence of this pericardial zone, it contributes in many cases of atrial infarction to a considerable reduction of the definitely necrotic central zones.

#### e. Foamy degeneration.

It has been mentioned, that in atrial infarction foamy degeneration of the myocardium usually dominates the histological picture, being the typical finding in lumen zones and pericardial zones.

Myocardial fibres in the state of foamy degeneration appear with most stains to be more or less empty of fibrils but yet swollen or inflated by some invisible substance. The remaining fibres are often found immediately under the sarcolemma; fibres cut transversely may consequently appear ring-shaped. All transitions may be found between normal fibres and fibres which seem to be nothing but empty sarcolemmal tubes. The cross-striation may be traced as long as there are any myofibrils left. The nuclei of the muscle cells are usually more conspicuous than in normal myocardium; sometimes they are bulky and inflated, sometimes they are flattened out, of irregular contour and staining darkly. The picture suggests, that such fibres are not completely necrotic, but in a more or less advanced state of degeneration.

It has been mentioned, that in ventricular infarcts foamy degeneration is usually found in a narrow marginal zone of the infarct area. In quite recent infarct cases the picture of foamy degeneration was often indistinct, but in a ventricular infarct of about 4 days standing it was fully developed. In lumen zones foamy degeneration may be seen together with all stages of development in the central zone. The observation of foamy degeneration in the lumen zone thus gives no clue to the age of the actual lesion.

It is possible that foamy degeneration represents sometimes only a stage in a process of slow disintegration of myocardial fibres. Such a development is however probably not the rule, at least not in the lumen zones. Foamy degeneration is a regular finding in the lumen zones of recent or subrecent infarcts, but a lumen zone consisting of living myocardium is practically always present also in old, scarred infarcts. It may be added that the myocardium is often histologically normal in the lumen zones of *old* infarcts. This observation argues for the assumption that foamy degeneration is an essentially *reversible* condition.

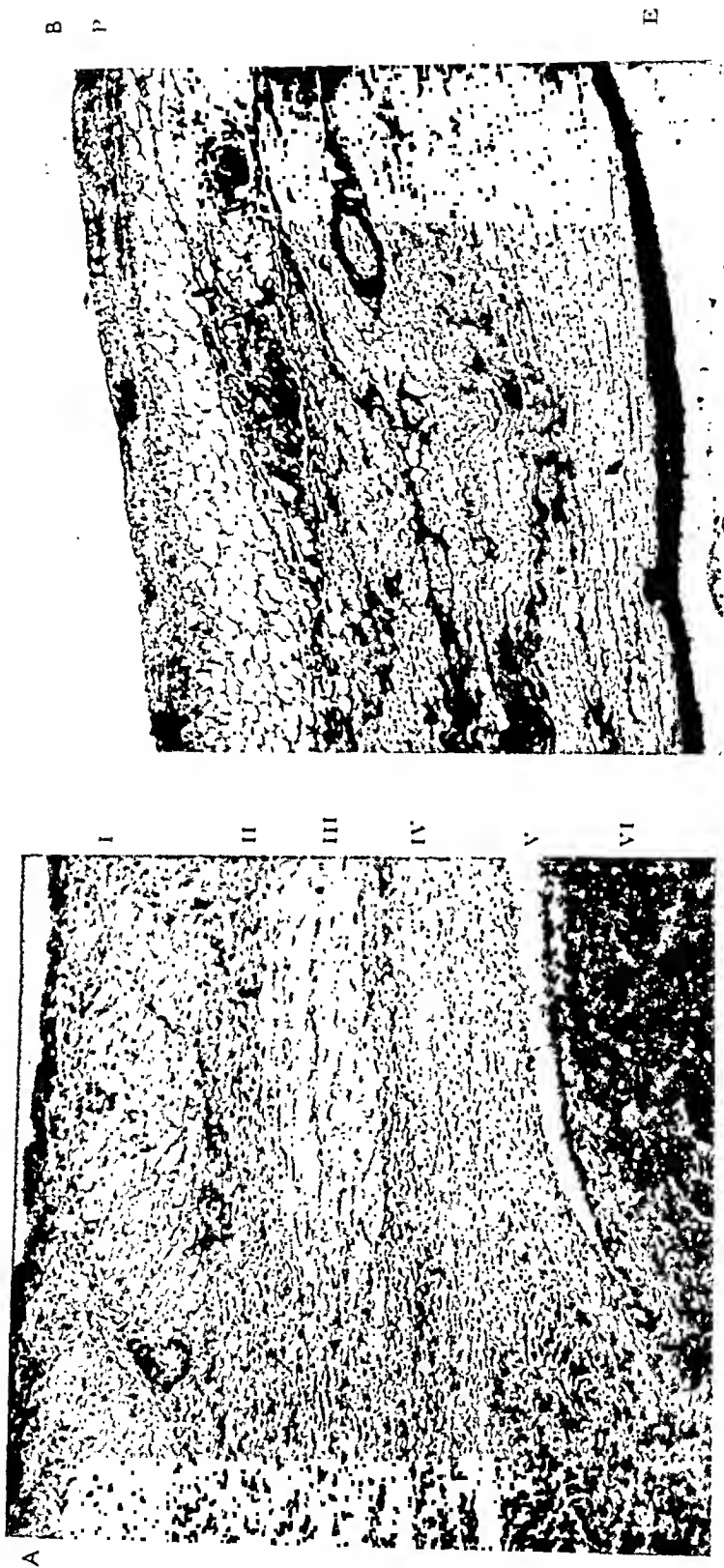


Fig. 17. A. Subrecent infarct in the left atrium. B. Recent infarct in the right atrium.

I: pericardial zone. Foamy degeneration. II: central zone consisting of new connective tissue. III: lumen zone, only reaching the margin of the free atrial lumen (V). IV: endocardium. V: free atrial lumen. VI: thrombus. Note the "capsular" organization. (Ser. A, case 137, v. Gleson  $\times 75$ ). B: endocardium. P: pericardium. Fibres with hyaline necrosis have taken a darker stain. Foamy degeneration dominates the picture (Ser. A case 133, v. Gleson  $\times 30$ ).



A



B

Fig. 18. A. Common picture of a recent infarct in the right atrium. Hyaline necrosis of a couple of fibres in the center of the trabeculum, foamy degeneration predominates. (Ser. A, caso 40, v. Gieson  $\times 80$ ). B. Recent infarct within old thrombus (right atrium). Hyaline necrosis throughout. No lumen zone. (Ser. A case 123 v. Gieson  $\times 30$ ).



Fig. 19. Infarct in the right atrium. Central scar in a small trabeculum, normal myocardium in the lumen zone. There is a "lumen zone" also around the trabeculum artery. (Ser. A, case 152, v. Gieson,  $\times 40$ ).

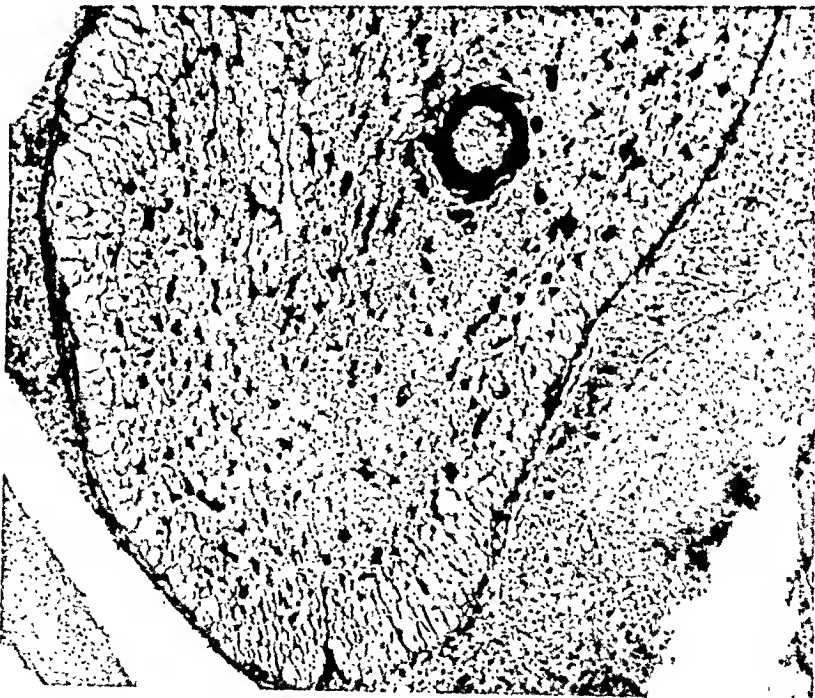


Fig. 20. Infarct in a trabeculum of the right atrium. Foamy degeneration in the lumen zone, which extends around a thin-walled vessel (or intertrabecular space), but not around the trabecular artery. (Ser. A case 130, v. Gieson,  $\times 80$ ).

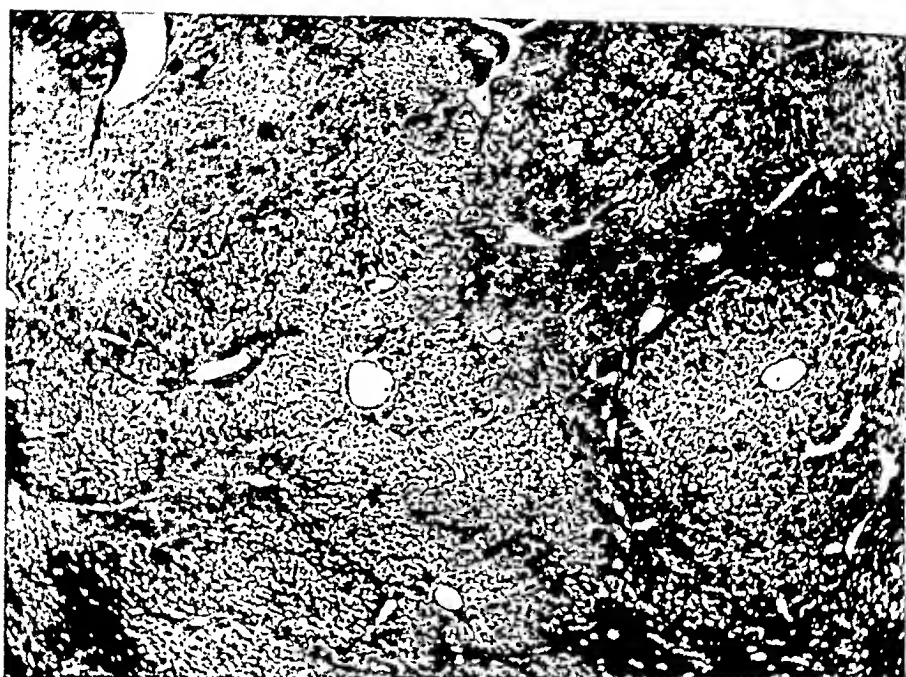


Fig. 21. Infarct of papillary muscle, left ventricle. Lumen zones surrounding thin-walled vessels. The dark areas represent scar tissue (the slide is stained with carmine and hematoxylin according to Best, but incompletely differentiated.  $\times 48$ ).

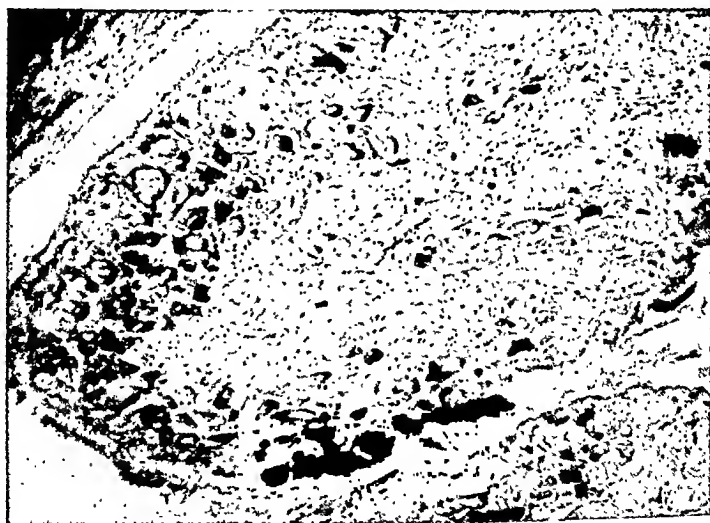
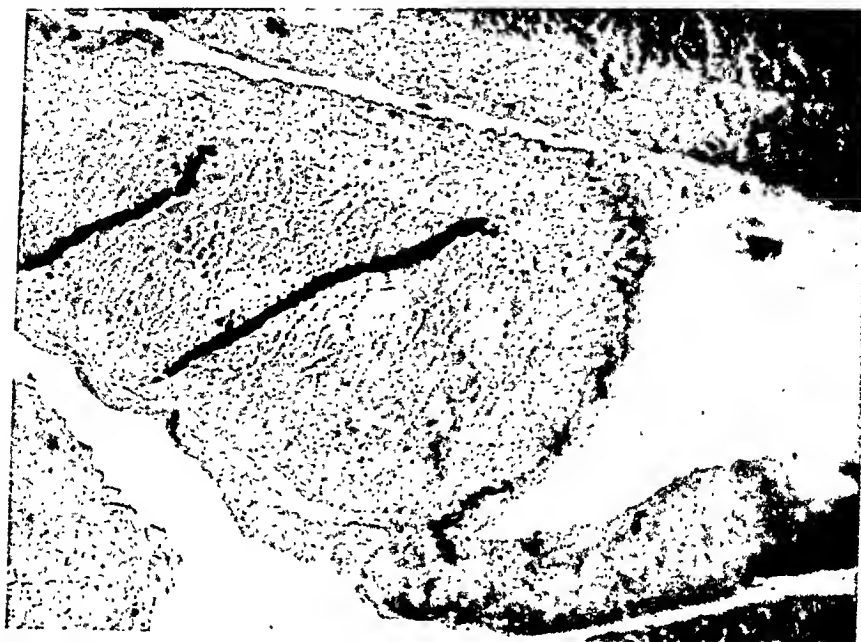


Fig. 22. Ventricular infarct. Old scar in the central zone, foamy degeneration in the lumen zone. The fibres are rich in glycogen. (Best's glycogen stain-hematoxylin,  $\times 100$ ).



A



B

Fig. 23. Recent infarct in the left ventricle. Foamy degeneration in the inner zone, which is rich in glycogen (black). Hyaline necrosis in the central zone. (A: v. Gieson. B: Best's glycogen stain-hematoxylin. Consecutive sections of the same trabeculum,  $\times 48$ ).



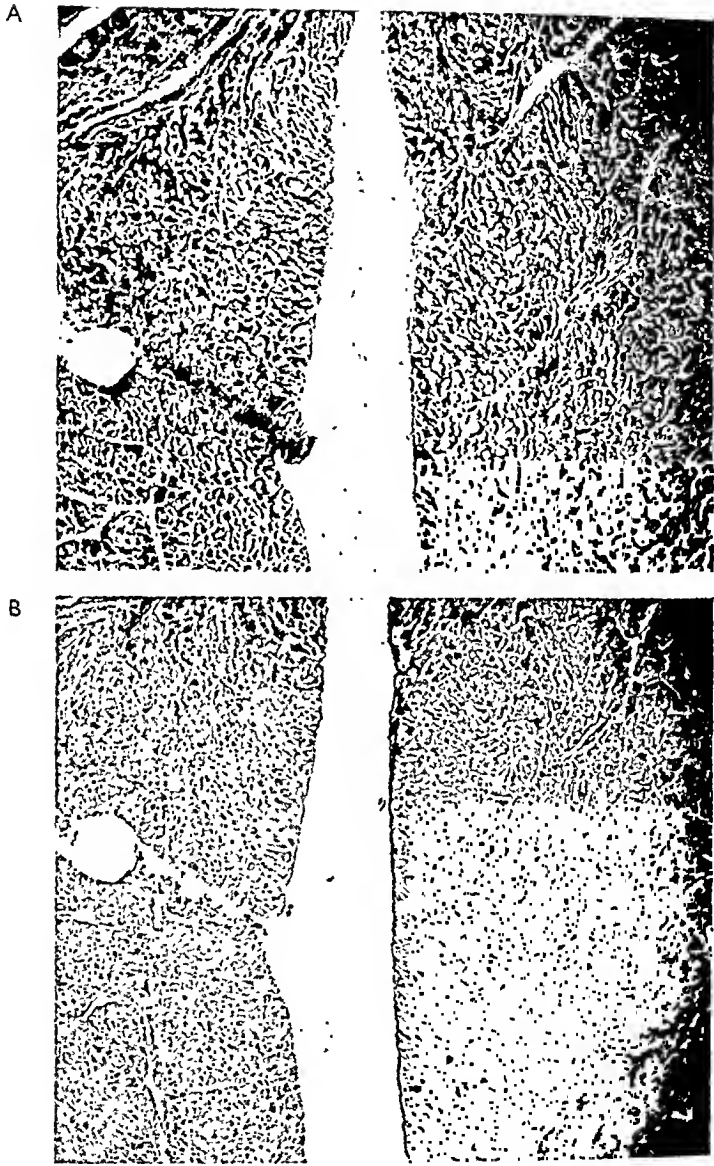


Fig. 24. Non-"coronary" foamy degeneration (diabetic glycogenosis myocardii). Note the complete absence of a lumen zone (A: Best's glycogen stain, B: v. Gieson, corresponding areas in consecutive sections.  $\times 48$ ).

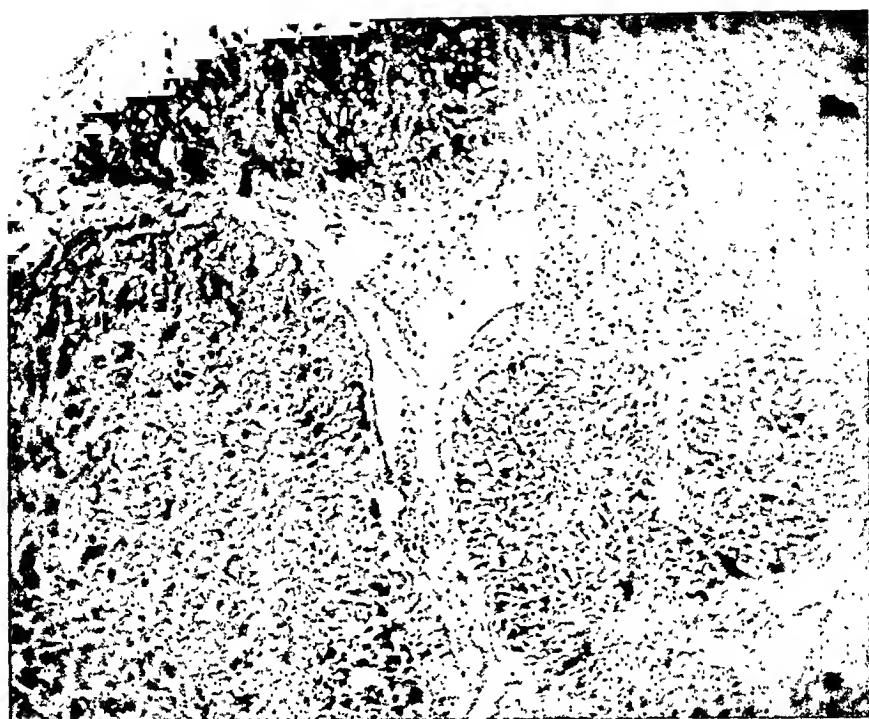


Fig. 25. Common picture in infarcts of the right atrium. Diffuse foamy degeneration and considerable amounts of glycogen in the fibres but no distinct differentiation of lumen zone and central zone. (Ser. A case 187, Best's glycogen stain-hematoxylin,  $\times 56$ ).



Fig. 26. Another area of the slide reproduced in fig. 25. A central zone of hyaline necrosis, devoid of glycogen, is distinctly differentiated in the lower part of the figure. This finding permits the diagnosis of "coronary" lesion. (Best's glycogen stain-hematoxylin,  $\times 30$ ).

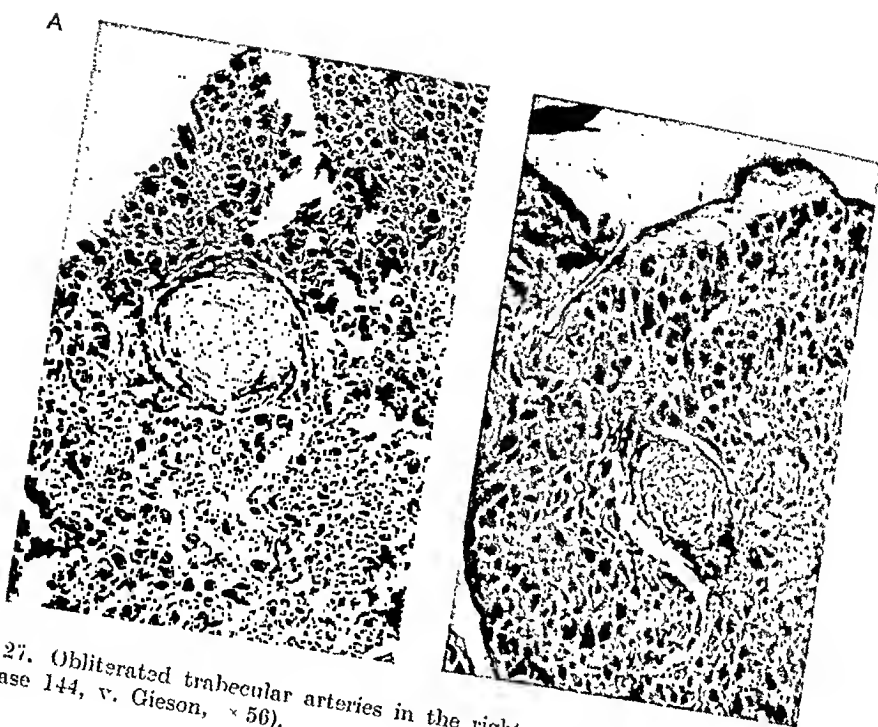


Fig. 27. Obliterated trabecular arteries in the right atrium (see text p. 72). (Ser. A, case 144, v. Gieson,  $\times 56$ ).

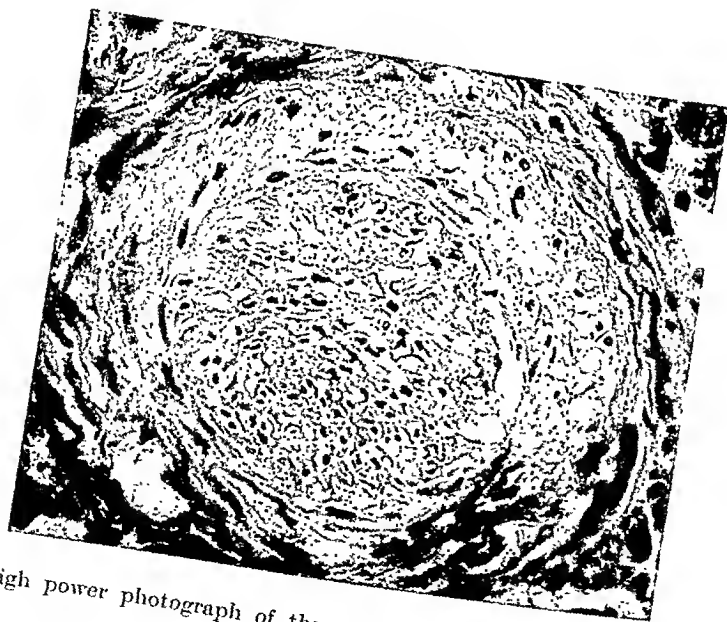


Fig. 28. High power photograph of the artery in Fig. 27 A.

The picture of foamy degeneration just described is almost invariably found in "coronary" lesions of the myocardium, but it is by no means diagnostic of a "coronary" etiology. A similar type of degeneration was described by WENCKEBACH in beri-beri hearts under the name of "hydropic degeneration" of the myocardium, and similar pictures have also been observed in diphtheric myocarditis and some other form of myocardial disease. It is only the finding of a differentiated lumen zone which is conclusive evidence of a "coronary" etiology. Fig. 24 is representative for a case of non-"coronary" foamy degeneration (diabetic glycogenosis of the myocardium) without a trace of a lumen zone.

In the investigations of PICHOTKA and ROTTER & MUELLER vacuolisation of myocardial fibres was observed as an immediate consequence of general hypoxemia (sudden death at high altitudes). The picture of their cases was evidently not quite the same as that of foamy degeneration. The present author can not decide to what degree the two conditions may be related to one another.

Finally FROTHINGHAM describes a vacuolar degeneration of the atrial myocardium (probably identical with the present author's foamy degeneration), which he assumed to be the morphological substrate of auricular fibrillation. In a larger material YATER failed to confirm the observation of FROTHINGHAM as to the relation between auricular fibrillation and this type of degeneration, but gives no further comment on the question.

#### f. The glycogen content of fibres in foamy degeneration.

At first the present author was inclined to regard the zone of foamy degeneration as identical with the zone of fatty degeneration, which is, (according to some writers), a regular finding at the borders of myocardial infarcts. However fat stains never revealed appreciable amounts of fat in the regions of foamy degeneration, and if fatty degeneration was observed, the myocardium of such areas did usually not present the picture of foamy degeneration. Impressed by the similarity between the typical PURKINJE fibres of the ungulates and fibres in less advanced stages of foamy degeneration, the author stained for glycogen, and found that *fibres in the state of foamy degeneration usually were rich in glycogen* (both in ventricular and atrial infarcts).

In most of the author's cases the autopsy had been performed 12—72 hours after death and no glycogen was demonstrable histologically in the normal fibres — it was also absent in the zones of hyaline necrosis. The glycogen was still plainly demonstrable in the zones of foamy degeneration from a case in which the autopsy was performed 48 hours after death. The glycogen was also found to be present in early stages of infarction in which a distinct picture of foamy degeneration was still not to be seen in the border zones. The staining for glycogen thus proved to be valuable in tracing "coronary" degeneration zones, which would otherwise be difficult to recognize.

It is well known, that the living myocardium is rich in glycogen, but also that it disappears quickly after death. BLUME found (in the hearts of dogs)  $3\frac{1}{2}$  hours after death about the half and 9 hours after death about  $\frac{1}{9}$  of the quantity of glycogen originally present. Exact figures for the disappearance rate of *histologically* demonstrable glycogen are not available. BERBLINGER found glycogen to be fairly regularly present in ordinary myocardium in autopsy material obtained 1—6 hours after death, but NAGAYO who obtained his material 5—12 hours after death found glycogen practically only in *specific* muscle fibres (particularly in PURKINJE fibres).

It is at any rate certain, that considerable amounts of glycogen are seldom demonstrable histologically in the fibres of ordinary myocardium if the autopsy has been performed more than 6 hours after death. The present author stained for glycogen in sections of atrial and ventricular myocardium from 46 cases selected at random among routine autopsies, performed 6—72 hours after death. Noteworthy amounts of myocardial glycogen were observed in 11 cases. A diffuse myocardial glycogenosis was observed in 2 of these cases, one case of diabetes mellitus (the occurrence of myocardial storage of glycogen in some diabetics is well known) and one case of cancer ventriculi with severe anemia. In the remaining 9 cases fibres rich in glycogen were found only in the marginal zones of ventricular infarcts (zones of foamy degeneration) but not in the normal (or in the completely necrotic) fibres.

It is a well established fact that the normal cellular stores of glycogen are rapidly reduced during hypoxia. This fact may account also for the disappearance of cellular glycogen after death. As a matter of fact GRAYZEL, TENNANT, STRINGER & SUTHERLAND

could state (by gross chemical analysis) that also the glycogen content of the *myocardium* is significantly lower in areas of experimental infarction than in the surrounding, normal parts of the heart.

The present author's experiences agree with those of GRAYZEL et al. inasmuch as glycogen is never present in the zones of hyaline necrosis. The marginal zones which are rich in glycogen are relatively narrow and often inconspicuous in ventricular infarcts. This glycogen must be difficult to detect with gross chemical analysis. The position is different in atrial infarcts, in which the zones of glycogenic foamy degeneration are usually quite dominant.

The facts presented regarding the behaviour of cardiac glycogen in "coronary" heart disease are thus to a certain degree contradictory. The occurrence of a conspicuous glycogen storage has been demonstrated in a certain type of myocardial lesion due to "coronary" insufficiency. On the other hand tissue hypoxia is known to promote a quick depletion of the cellular stores of glycogen. The author will give a more detailed survey of this problem in a coming paper and refrains from a complete discussion of the matter here. However it may be pointed out that all fibres which are rich in glycogen (v. GIERKE's disease, coronary and diabetic glycogenosis, rhabdomyoma fibres and normal PURKINJE fibres) have in common that they are poor in myofibrils. This fact may account for a reduced glycogen consumption. If the power of accumulating glycogen were intact, conditions for a glycogen storage would be present in such fibres.

As early as 1883 EHRLICH observed in 2 cases of myocardial infarct that the centres of the infarcts were surrounded by broad zones of fibres rich in glycogen. EHRLICH studied the glycogen distribution in cases of diabetes mellitus and noted the finding only as a source of error. In modern literature the phenomenon seems to have been quite neglected. It is however mentioned in the paper of ANTOPOUL et al. that "it is known that after severe myocardial ischemia the venules, as a rule, contain glycogen".

Pathological accumulation of glycogen is otherwise best known from the glycogen storage disease of v. GIERKE. In typical cases of this disease glycogen storage is most conspicuous in the liver but it is usually also present in the heart. PUTSCHER was the first to describe cases of this disease in which glycogenosis of the heart was the dominating feature. An increasing number of similar cases have

been described later (see ANTROPOL, BOAS, LEVISON and TUCHMAN). The histological picture is described as very typical even without staining for glycogen. Thus POMPE reexamined the slides from some cases which had previously been labelled "idiopathie cordiae hypertrophy" and recognized them as belonging to this group. On the other hand, according to microphotos reproduced, *this typical histological picture seems to be impossible to distinguish from the present author's foamy degeneration.*

The glycogen storage in the myocardium of diabetics has already been mentioned. Little is known about the occurrence and importance of this condition. Finally heart tumours of the rhabdomyoma type are known to contain large quantities of glycogen.

A case described by FINKELSTEIN under the diagnosis of *cardiomegalia glycogenica circumscripta* is of special interest in this connection.

In this case, the left coronary artery originated from the pulmonary artery and there was a hypertrophy specially affecting the parts of the heart nourished by this artery. The histological picture of this hypertrophic myocardium presented a mixture of fibrous scars, recent necrosis and large areas of myocardium with an appearance quite similar to the present author's foamy degeneration. Glycogen was abundantly present within these degenerated fibres. In several other cases of pulmonary coronary artery (e. g. 2 cases described by SCHOLTE) the same picture of foamy degeneration was found but staining for glycogen had not been performed.

Another case of *cardiomegalia glycogenica circumscripta* was described by v. CREVELD and v. D. LINDE in a child, 5 months old. In this case there was a congenital dilatation of the pulmonary artery, the origin of the coronary arteries was not mentioned, but in the microphotos reproduced a distinct differentiation into lumen zone and central zone is plainly visible, suggesting a "coronary" etiology for the myocardial degeneration observed (the present authors comment).

The present author finds it probable that the *cardiomegalia glycogenica circumscripta* of FINKELSTEIN and v. CREVELD & v. D. LINDE simply represents a state of circumscribed myocardial degeneration due to "coronary" insufficiency (myocardial infarction).

### Summary.

The histological picture of atrial infarcts is in many respects different from the one in ventricular infarcts. Areas of complete necrosis

are often inconspicuous and present only in the central parts of the trabeculae. Most of the atrial myocardium falls within zones beneath the endocardium (lumen zone) and the pericardium (pericardial zone) which are sheltered from the extreme consequences of failing coronary blood supply. The occurrence of a lumen zone is diagnostic for a "coronary" etiology of the lesions.

At least in right atrial infarcts these zones usually present a type of myocardial degeneration, which has been called by the present author foamy degeneration. Myocardial fibres in foamy degeneration are poor in fibrils and *rich in glycogen*. The author suggests, that some cases previously described under the diagnosis of cardiomegalia glycogenica circumscripta exemplify "coronary" degeneration zones of this type.

## CHAPTER VIII.

### A classification and general analysis of the material of atrial infarcts.

#### a. Extension and age of the lesions.

It is evident from the account in chapter VI, that atrial infarcts do not usually attain the massive size of ventricular infarcts. In atrial infarcts, zones representing quite different degrees of myocardial damage alternate continuously in the same slide, and the wide and monotonous "deserts" of dead myocardium, typical of infarcts in the left ventricle, are seldom seen. One might be tempted to assume quite another pathogenesis for the mild lesions which often dominate the picture of atrial infarcts. It may be concluded, however, from the discussion in chapter VI, that the difference is nothing but a reflection of the fact that atrial myocardium is not so absolutely dependent on the blood supply through the coronary arteries as ventricular myocardium. The term myocardial infarct is yet equally applicable to both types of lesion.

In this series diagnosis was based on histological findings. As a natural consequence the cases represent all transition stages between extensive infarcts and minute foci of degeneration. A corresponding, continuous series is present also with respect to the age of the lesions.



Some sort of classification had however to be applied to the material in order to make a further analysis possible. In the absence of natural dividing lines the author has classified the material arbitrarily according to the following principles:

1. *Extension (and degree) of the lesions.*

The author must point out, to begin with, that an exact conception of the extension of atrial infarcts is to be obtained only from serial sectioning, a procedure which has been performed in none of the author's cases. According to the extension of the lesions within the sections available, the author has however attempted a division of the material into 2 main groups, termed "infarcts" and "minor lesions".

Atrial *infarcts* were considered to be present if there was *hyaline necrosis or homogenous scars in the central zones* and if the more or less contiguous lesions covered areas of *reasonable extension* in the slides (order of size in centimeters).

The term *minor lesions* was used for solitary or sparsely disseminated small foci, presenting the stigma of "coronary" lesion (differentiation into lumen zone and central zone), but too small to justify the expectation of clinical signs (order of size in millimeters). Cases, in which there was no complete necrosis in the central zone, were also placed within this group (regardless of the extension of the lesions).

If these principles of classification are applied to the author's material of atrial lesions, it may be described as follows:

In series A 46 cases of *atrial infarct* were found. In addition the author observed atrial infarcts in 1 case without mural thrombi. *The total number of atrial infarcts observed in this study is consequently 47.* The infarcts were found, *in 46 cases* in the walls of the *right atrium* and only *in 1 case* in the walls of the *left atrium*.

*Minor lesions* were observed in series A *in 33 cases.* They were found in the walls of the right atrium in 31 cases, in the walls of the left atrium in 1 case and in the walls of both atria in 1 case.

The limit between "infarcts" and "minor lesions" is obviously rather indistinct. The author's only purpose in grouping them thus was to segregate from the main material all cases (minor lesions) which seemed too inconspicuous to merit special attention with respect to the clinical signs.

## 2. Age of the lesions.

It is obvious from the discussion in chapter VI that the age of the lesions may be judged only from the appearance of the *central* zone. The actual state of the lumen zone may have little to do with the disturbance of the coronary blood supply which once induced the lesion.

Infarcts in which the *central zone* consisted of *mature scar tissue*, rich in collagen and poor in cells have been labelled *old infarcts*.

*All other infarcts* were labelled *recent*, even if the necrotic tissue of the central zone had been replaced by scar tissue, if this tissue was still rich in cells and vessels. The term *subrecent* will sometimes be used for such not quite recent lesions.

It was not exceptional to find in the same case areas of both old and recent infarct, sometimes alternating in the same slide. In the *infarct* material there were 7 cases of exclusively old infarct, 13 cases of mixed old and recent infarcts and 27 cases of recent infarct.

### b. The immediate causes of atrial infarcts.

Recent, occluding thrombi in the main coronary arteries may have been directly responsible for the atrial infarcts observed in 10 cases (the thrombus was found 9 times in the right coronary and once in the circumflex branch of the left coronary). In 1 case there was a recent occlusion of the interventricular branch of the left coronary artery; it is difficult to decide if this finding had anything to do with the occurrence of atrial infarction. In 30 of the remaining cases coronary arterial sclerosis was prominent, usually with calcification of the arterial walls and sometimes distinct stenoses, but no actual occlusion. In 14 of these cases old ventricular infarcts and in 6 cases recent ventricular infarcts were also recorded.

Changes in the atrial branches of the coronary arteries may have caused the atrial infarcts in some of these cases. The author has not searched systematically for the presence of obstructions in the atrial arteries. The arteries visible in the sections available usually presented little of actual interest in this respect.

In some cases, however, (also in several cases without demonstrable infarcts) actually obliterated arteries were observed in the sections. They were usually trabecular arteries, the external diameter of which was 0.2—0.6 mm, as a rule several obliterated arte-

ries were observed in the same case. In some cases the arterial lumen was filled up by a solid scar tissue, suggestive of an organized thrombus. In other cases the lumen seemed to have been occupied by a mass of small, clear cells, similar to smooth muscle cells cut transversely. The author saw this type of (multiple) arterial occlusion in several cases (the microphotos in figs. 27, 28 are from case 144). The author believes personally that this picture represents a special type of coronary arterial disease but refrains from expressing any opinion of his own regarding its etiology and importance. The picture was definitely *not* similar to any of the types of endarteritis described by several authors (v. GLAHN & PAPPENHEIMER, KARSNER & BAYLESS, GROSS, KUGEL & EPSTEIN and others) especially in rheumatic heart disease. It is instead somewhat reminiscent of the apparatus present normally in some special arteries (renal arteries, arteries of arterio-venous anastomoses, bronchial arteries — as a matter of fact also in some coronary arteries, according to BUCHER, 1945) which has been assumed to render possible an active occlusion of such arteries (as a physiological activity).

In 7 cases the coronary arteries were quite normal or there was at least only inconsiderable atheromatosis.

Two of these cases (Nos 144 and 133) had rheumatic heart disease (severe aortic stenosis). In both these cases multiple obliterated, small arteries were observed in the atrial walls.

In 2 other cases (Nos 170 and 129) the immediate cause of death was systemic arterial embolism (left coronary artery in case 129, right femoral artery in case 170). Arterial embolism may in these cases have been the cause for the atrial infarcts too.

In the 3 remaining cases (Nos 32, 83 and 130) no immediate causes were found for the atrial infarcts observed. Old ventricular infarcts were present in 2 of these cases.

It must be noted here, that most cases of atrial infarction had shown definite signs of congestive heart failure for a long time before the onset of the terminal illness. This contrasts with the experience that a past history of congestive heart failure is comparatively seldom obtained from cases of acute ventricular infarction. In 9 of the cases of atrial infarction had congestive heart failure not been prominent in the past. In 8 of these cases large, acute ventricular infarcts were the main cause of death. Isolated atrial infarcts occurred exclusively in cases with long-standing congestive failure. The reason may be, that the stress placed on the atrial myocardium by

high intra-atrial pressure promotes the development of infarcts according to the mechanism discussed on page 10.

c. The general frequency and distribution of atrial infarcts.

It has been generally accepted previously, that atrial infarcts are of rare occurrence. CUSHING and his coworkers maintain an opposite opinion, noting atrial involvement in 17 per cent of all cases of myocardial infarct.

The author does not know, if the *ventricular* myocardium in the material of CUSHING et al. was subjected to the same close scrutiny (histologic examination) as the atrial myocardium. In the present author's investigation this was certainly *not* the case. It is probable, that many ventricular infarcts, of an order of size comparable to the present author's atrial infarcts, may escape notice on gross inspection. A comparison of the frequencies of atrial and ventricular infarcts observed in the present material would consequently not give a correct conception of the relative frequency of atrial infarcts.

The present author's material of atrial infarcts was obtained with atrial thrombosis as a primary clue. It includes only one case *without* mural thrombi. As a matter of fact, cases of atrial infarct without atrial thrombosis are probably not common. The present author has during the course of this investigation examined about 50 cases without atrial thrombi, in which a suspicion of atrial infarction seemed justified for other reasons, but only the single case mentioned was found. It is however probable, that mural thrombi do not appear immediately after the onset of infarction. MALLORY et al. did not observe mural thrombi before the fifth day after the onset of ventricular infarction. It may be added, that in the series of CUSHING et al. mural thrombi were registered only in 26 out of 31 atrial infarct cases. The present author's figures for the general frequency of atrial infarcts are thus probably somewhat too low; a number of very recent infarcts in particular may have escaped notice (such very recent infarcts are, in addition, difficult to recognize even under the microscope).

The following considerations may, however, give an idea of the general frequency of atrial infarcts. In series A infarcts were found in about 1/3 of all cases with right atrial thrombi. In the large autopsy material of series B right atrial thrombi occurred in about 3 per cent

of all cases. Consequently *atrial infarcts are to be expected at least in about 1 per cent of all autopsies* in a general autopsy material of the Uppsala type. The figures refer only to right atrial infarcts, left atrial infarcts being too rare to exert any influence on the calculations.

The rarity of infarcts in the left atrium is a somewhat surprising phenomenon. Of the total number of atrial infarcts published before the present study, 52 occurred in the right and 11 in the left atrium, and in the present author's series there is only *one* leftsided infarct against 46 rightsided ones.

To explain this distribution picture LANGENDORF (who knew 1 leftsided and 3 rightsided atrial infarcts) pointed out, that an early obturation of the right coronary may shut off all the branches from this artery to the right atrium, and the subject may yet survive sufficiently long to permit a demonstrable infarct to develop. A corresponding obturation of the left coronary would in most instances be followed by a sudden death. LANGENDORF is obviously thinking of thrombosis in the left coronary *before its bifurcation*, though this need not necessarily occur, as the ramus atrialis sinister anterior according to SPALTEHOLZ usually originates from the circumflex branch. The relative rarity of thrombi in the circumflex branch of the left coronary may, according to LANGENDORF, work in the same direction.

CUSHING et al. suggested that the high oxygen content of the blood in the left atrium might be of importance. The present author is prone to regard this factor as the most important one. It has been mentioned that, but for the bad nutrition of the lumen zone, it would probably be difficult to detect infarcts also in the *right* atrium. It is furthermore probable, that the lumen zone in the left heart is generally broader than that in the right heart. It is finally evident that minor lesions (due to local disturbances of the coronary blood supply) would not be so rare in the left atrium if there were not another factor than the distribution of the coronary arteries to prevent the appearance of such lesions.

#### d. The distribution of infarcts within the right atrium.

Only for a restricted number of cases can *complete* data be given for the distribution of the infarcts within the walls of the right atrium.

The reason is, again, that the lesions could be recognized with certainty only under the microscope, and that the blocks during the first period of the investigation were taken with the distribution of mural thrombi as a main guide. A convenient procedure for the systematical (gross) localisation of atrial infarcts was established during the course of the work; it proved desirable to have sections representative of at least the following 4 regions: I. the auricular appendage. II. the ventromedial part of crista terminalis (with the sinus node and "antrum"). III. the lateral wall. IV. the dorsal wall + the dorsal half of septum atriorum. The author has seen sections from all these regions in 26 infarct cases.

It is nevertheless possible to distinguish in this material at least 2 main distribution types of atrial infarcts.

1. Infarcts in the dorsal wall of the right atrium (region IV), to be called *dorsal infarcts* in this study. In the author's series there were 14 cases of dorsal infarcts. In 6 cases the infarct was restricted to the dorsal wall, in the remaining cases lesions were found in other regions as well. In 12 of these cases there was also a recent "posterior" infarct of the left ventricle and in 7 cases a recent, occluding thrombus of the right coronary was probably the immediate cause of both the ventricular and the atrial infarct. The dorsal infarct of the right atrium can thus usually be regarded as a continuation in the atrial walls of the classical "posterior wall" type of ventricular infarct.
2. In the remaining cases, the main lesions were usually found in the walls of the auricular appendage, extending more or less into the antrum or the lateral wall. Unfortunately, sections to *prove* the freedom of the dorsal wall from infarction are only available in 12 of these 33 cases. The homogeneity of this group is thus somewhat questionable, the author will however use the term *ventral infarct* for this distribution picture. One fact of interest must be stressed. Ventricular infarcts, of approximately the same age as the atrial infarcts, were present only in 4 cases (3 "anterior wall" and 1 "posterior wall" ventricular infarcts). In most cases there was no evidence of recent lesions in the ventricular myocardium.

It is a somewhat surprising fact, that infarcts in the large myocardial mass of crista terminalis (cranial part) were found only in

4 cases. The myocardium of crista terminalis sometimes seemed to be spared in cases with extensive infarcts, including most of the neighbouring structures. The same can be said about the sinus node which was observed in a considerable number of cases. It must be pointed out, that the judgment as to pathological changes of the sinus node is very difficult. This node normally looks very like a fibrous scar, containing scanty remnants of myocardial fibres. "Scarring of the sinus node" is a finding often referred to in the literature as a morphological substrate for disturbances of the sinus rhythm. A relative increase in the amount of fibrous tissue was probably present in some of the sinus nodes observed (case No. 183 may be mentioned as an example) but the myocardial fibres of the node were never seen to be totally absent or present considerable signs of degeneration.

### Summary.

The author observed 47 cases with extensive "coronary" lesions in the atrial myocardium (labelled "atrial infarcts") and 33 cases of less extensive "coronary" lesions (labelled "minor lesions"). Only the cases with atrial infarcts have been the object of a further analysis.

Most cases reported in this investigation had recent infarcts. Recent and old infarcts were however often present in the same case.

Occluding thrombi in the main coronary arteries were in a restricted number of cases probably responsible for the atrial infarcts observed. In most cases actual obturations of the coronary arteries were not observed.

Atrial infarcts were observed in 46 cases in the right atrium and in one case in the left atrium. The author is inclined to attribute the rarity of left atrial infarcts mainly to the high oxygen tension in the blood within the lumen of the left atrium.

The author observed 2 main distribution types of right atrial infarcts: the *ventral* type represented by usually isolated infarcts in the auricular appendage and adjacent parts of the atrial wall and the *dorsal* type, to be regarded as the atrial part of a large infarct area, the main part of which was represented by "posterior wall" infarcts of the ventricles.

## CHAPTER IX.

**Symptoms of atrial infarcts during life.****a. General considerations.**

It was stated in chapter VII that atrial infarcts (if searched for) are not exceptional findings at autopsy. They are usually not very conspicuous lesions. In most cases there was little reason to assume, that the atrial infarcts were fatal in themselves. The conclusion may seem justified, that most subjects contracting an atrial infarct survive. Consequently atrial infarcts might be expected to be rather common in a clinical material.

An objection may be raised against this reasoning. In the autopsies atrial infarcts often have the character of complications of other affections (e. g. ventricular infarcts) which are to be regarded as main causes of death. It is possible that atrial infarcts mainly occur in such combinations, sharing the bad prognosis of the main disease. In addition it may be mentioned, that signs of vitiated atrial function are said to indicate a bad prognosis in cases of ventricular infarct. Auricular fibrillation is thus definitely an unfavourable sign in cases of ventricular infarct (MASTER, DACK & JAFFE, ASKEY & NEURATH) and it has been mentioned, that BLOOM & GILBERT attributed a similar importance to high P-waves.

Old, scarred infarcts are however common in the present author's material, and they would probably have been still commoner, were it not for the quick metamorphosis of fungiform thrombi, the remnants of which are usually hardly recognizable with the naked eye. Consequently the author believes that atrial infarcts should often be met with in clinical work. Signs to be attributed to atrial infarcts have however seldom been recorded previously. There are two possible explanations of this fact: the signs of atrial infarcts must either be very inconspicuous or manifest themselves in a quite unexpected way.

There are some *a priori* reasons for the first assumption. It must be stressed once again, that atrial infarcts seldom attain the size of ventricular infarcts. Ventricular infarcts corresponding in extension to most atrial ones present a clinical picture which is not clearly defined. Cases presenting only such (usually neglected) lesions are



as a rule classified clinically into the somewhat diffuse diagnostic groups of "coronary insufficiency" or "coronary sclerosis". The small, disseminated infarcts regarded by BUECHNER and his school as the anatomical substrate of simple attacks of anginal pain, correspond well in size to some of the present author's atrial infarcts of the disseminated type.

Consequently, there is little reason to expect considerable *general reactions* (e. g. fever, leucocytosis, hyperglycemia etc.) in cases of atrial infarction. Such signs will be described very briefly in the following survey. The clinical diagnosis of atrial infarction must probably be based exclusively on local signs from the atria themselves (e. g. disturbances of the heart rhythm and changes in the atrial part of the ECG).

Unfortunately, the author had to start from an autopsy material and to search *post mortem* for clinical signs in the hospital records. The information gained in this way was often very defective for the purpose in hand. Special diagnostic methods could not be employed, as it was only during the later period of the investigation that the author was able to define any criteria which might justify a suspicion of atrial infarcts *ante mortem* and even these were seldom satisfied. Most of the clinical findings discussed in this chapter are thus observations in solitary cases, and usually the author can only suggest, but not prove that they were signs of the atrial infarcts observed at the autopsy. They may however be used as starting points for future contributions to the symptomatology of atrial infarction.

#### **b. The past history, the immediate complaints and the general clinical picture.**

It has been mentioned that extensive coronary sclerosis was present in most cases of atrial infarct. It may be added, that most of them had also a definite arterial hypertension (hypertension was considered to be present if the systolic pressure was higher than 200 mm Hg or the diastolic pressure higher than 100 mm Hg in one reading). It can be said generally that there was nothing to distinguish the past histories in the atrial infarct cases from those of average cases belonging to the diagnostic groups of coronary sclerosis and hypertensive heart disease.

There was some justification for distinguishing two types of past

history in the material of atrial infarct cases. Most cases had a history of long-standing, congestive heart failure (in 29 cases *severe* congestive failure with high grade dyspnea, liver engorgement or manifest peripheral edema had been definitely present for at least 6 months before death). Most of these cases had "ventral" infarcts, usually situated in the auricular appendage, and no recent ventricular infarcts. In a smaller group there had been little or no signs of congestive failure previously (9 cases), of these cases 8 had "dorsal" atrial infarcts, to be regarded as continuations of coexisting ventricular infarcts of the "posterior wall" type.

It is possible that the use of mural thrombi as indicators of atrial infarcts is responsible for a too high incidence of advanced congestive failure in this material. There is, at any rate, little reason to attribute the presence of congestive failure to the atrial infarcts, a reverse causal sequence is more probable. In some cases a rapid aggravation of the congestive failure coincided with the onset of auricular fibrillation some weeks before death. The part which atrial infarcts may have played in this course of events will be discussed later on.

As a regrettable effect of the post mortem character of this material the *immediate complaints*, which brought the patient to the hospital, could usually not be directly attributed to the atrial infarcts. The same can be said about the main features of the clinical syndrome ante mortem. As regards the dominating clinical picture the cases can be classified in the following way:

Congestive heart failure 16 cases

Syndrome of acute ventricular infarct 16 cases

Cerebral accidents (usually cerebral embolism) 5 cases

Other systemic embolism 2 cases

Pulmonary embolism 4 cases (Pulmonary infarcts were present as less striking findings in 3 additional cases with congestive failure)

Uremia 2 cases

Lung abscess 1 case

Myeloma 1 case

If there existed any independent clinical syndrome of atrial infarction it obviously had but a little chance of making its voice heard in the mighty symphony of such alarming and impressive terminal

syndromes. Some general signs, which are known to be helpful in the diagnosis of ventricular infarcts (fever, leucocytosis, raised sedimentation rate, hyperglycemia), were present in many cases of atrial infarct, but there is no case in which they could not be attributed to other coexisting disease as well. It is consequently of no purpose to give a complete account of the occurrence of such signs.

Persistent anginal pain of the classical infarct type was recorded only in cases with coincident ventricular infarcts, and there was nothing peculiar in these cases about the site of the pain or with its irradiation. Some cases without recorded ventricular infarcts had experienced transitory anginal pains (usually with the character of effort angina) during the months before death, but there is no special reason to assume, that these pains were necessarily derived from the atria. On the other hand, in 20 cases of atrial infarcts, many of which had extensive lesions, nothing is mentioned about anginal pains in the reports. It thus seems justifiable to assume, that anginal pains do not constitute an important feature in the clinical picture of atrial infarction.

As a matter of fact it was often possible, on going through the hospital records, to note some incident, which *may* have been related to the onset of the atrial infarct (rise in temperature or heart rate, diffuse pains, usually in the epigastrium or an unexpected aggravation in the general state of the patient). But no such sign occurred with sufficient constancy to merit special mention as a probable sign of the onset of the atrial lesions.

### c. Atrial infarcts and auricular fibrillation.

Local signs would be expected to give a better clue for the diagnosis of atrial infarcts. Owing to the remote position of the atria such local signs are to be expected only in electrocardiograms. There is however one sign of disturbed atrial function which *may* be diagnosed with some degree of accuracy even without ECG's, viz. *auricular fibrillation*.

It is commonly accepted, that ventricular fibrillation is the cause of sudden death in many cases of ventricular infarction. As a consequence it may seem reasonable to expect, that *auricular fibrillation* may be a consequence of *atrial infarction*. CUSHING et al. include auricular fibrillation in the "abnormalities of the auricular

meehanism", which represent, according to these authors, the most reliable clue to the diagnosis of atrial infarction. The present author has made the same suggestion in a previous paper (1945).

It is important to realize in this connection, that auricular fibrillation in itself is a purely *functional* disorder. One may expect that the effect of atrial infarcts might be only an increased *disposition* for auricular fibrillation, and the actual development of fibrillation must not be an *immediate* consequence.

Auricular fibrillation was however surprisingly seldom present in the cases of atrial infarction published previously. Among 33 cases in the literature, in which the heart rhythm was noted, only 11 (33 per cent) had auricular fibrillation. This is no high incidence, compared with the fact, that in the present author's series B auricular fibrillation was present in 39.3 per cent of all cases dying of heart disease. It may be added, that a real auricular fibrillation has never been observed as a consequence of experimental atrial infarction.

In the present author's material of atrial infarcts, the incidence of auricular fibrillation is rather high. If some cases are included, in which the diagnosis of auricular fibrillation was made clinically (without ECG), transitory or permanent fibrillation was observed before death in 26/47 or 55.3 per cent of the cases. It must however be pointed out, that among 60 cases with right atrial thrombosis, in which no infarction or even "minor lesion" was found, 34 or 56.7 per cent had auricular fibrillation. The high incidence of auricular fibrillation in the present author's material of atrial infarcts *may* simply be due to the fact that it was collected mainly on the basis of the presence of atrial thrombi.

It has been mentioned, that chronic congestive heart failure was present in most cases of atrial infarction. There is consequently reason to believe, that many atrial infarcts develop in cases with pre-existing, chronic fibrillation.

In order to segregate such cases the author divided the cases with fibrillation into 2 groups. If fibrillation (as a constant or transitory condition) had *appeared* during the last 2 months before death it was defined as *acute fibrillation*. If it had been constantly present (to judge from the hospital records) during more than 2 months before death it was labelled *chronic fibrillation*.

According to these definitions *chronic fibrillation* was present with certainty in 12 of the infarct cases; in most of them it had pro-

bably been present for several years. Though old infarct scars were found in some of these cases, auricular fibrillation could at any rate not be regarded as a sign of the recent infarcts present in most cases belonging to this group.

In 4 cases no information was available as to whether the fibrillation was acute or chronic.

In the remaining 10 cases could it be established with certainty that the fibrillation had first appeared during the last 2 months before death. Thus, of 31 cases which probably had regular rhythm before the infarction 10 (32.3 per cent) developed auricular fibrillation during the following 2 months, *possibly* as a consequence of the infarcts observed post mortem. Corresponding figures for the cases with right atrial thrombi but without infarcts in the atrial walls were: 32 cases starting with regular rhythm, 6 of which developed auricular fibrillation (18.8 per cent). There is thus a higher incidence of recent auricular fibrillation in the series with infarcts than in the series without infarcts but the difference is not statistically significant ( $13.5 \pm 10.9$  per cent). It is possible that a significant difference could be demonstrated in a larger material.

It would be interesting to know if a disposition for auricular fibrillation may be related to the localisation of the infarcts. The author's material is too small to permit safe conclusions on this question. It may however be mentioned, that auricular fibrillation developed in 7 out of 19 cases labelled as *ventral* infarcts and in 3 out of 12 cases of mainly *dorsal* infarcts.

The author concludes from this discussion, that atrial infarcts may, in some instances, induce auricular fibrillation, although *significant* evidence for this assumption is not to be obtained from the present material. It is at any rate evident, that auricular fibrillation is a sign of little practical value for the diagnosis of atrial infarction during life.

As a matter of fact, auricular fibrillation did *not* develop in 2/3 of the cases, which had regular rhythm at the time for the onset of the infarction. Atrial infarction may thus be one of the factors responsible for the (relatively) high incidence of right atrial thrombi in cases with regular sinus rhythm.

#### d. ECG findings in the cases with atrial infarcts.

One could finally expect to find some conclusive signs of the atrial infarcts in the ECG's. Unfortunately, ECG's in which such signs

were really to be expected are available only in a restricted number of cases.

In 11 cases no ECG had been taken. In the remaining cases ECG's from the last 2 months before death are available in 32 cases. In 16 cases only one ECG had been taken during this period, in 16 cases several ECG's had been taken and in 14 cases ECG's are available both from the terminal period mentioned and from earlier periods. In 11 cases auricular fibrillation only had been registered. These ECGs can obviously offer little of interest for an analysis of the atrial part of the ECG (an exception is case 27, which will be commented on under the heading Picture of "double command"). There remains thus 31 cases, in which ECG's with visible P-waves are available from the last 2 months. Lesions, which may have arisen during these 2 months before death, were present in all these cases except for one (case 183) in which exclusively old lesions were found. This case is to be described under the heading "Duration of the P-Q interval".

In most ECG's little of special interest could be observed. Observations of some importance were, however, made in a restricted number of cases. They will be described individually in the following paragraphs.

### *Picture of "double command"?*

In case 27, ser. A, hypertension and congestive heart disease had been present at least 5 years before death. The patient died with the clinical picture of congestive heart disease. Autopsy showed left ventricular hypertrophy, moderate coronary atheromatosis, an infarct scar in the dorsal wall of the left ventricle and a mainly recent infarct in the trabeculated part of the right atrium ("ventral infarct"). Auricular fibrillation had been recorded 5 years before death. In an ECG taken 2 days before death there is apparently also auricular fibrillation but in addition a series of small, P-like deflections are registered occurring with rather regular intervals (about 0.8 sec.) quite independent of the ventricular complexes (fig. 29).

The picture suggests the possibility of a "double command" of the type described by CONDORELLI (1928) as a consequence of ligation of the interventricular branch of the left coronary in dogs. CONDORELLI observed sinus rhythm in the right atrium, coexisting with fibrillation in the left atrium. He assumed that the phenomenon was due to a deficient coronary blood supply to certain myocar-

dial structures in the atrial septum, uniting the myocardium of the right and the left atrium.

The author is, however, always wary of such "double command" ECG's in man. They are often only artefacts, occurring if some other person touches the patient when the ECG is recorded. The ventricular complexes of the attendant may then appear as an independent series of "P"-waves in the ECG. There is no special reason to assume such a mechanism in this case but the author was not present when the ECG was taken and cannot exclude this possibility.

### *Disorders in the atrial rhythm.*

Apart from auricular fibrillation, atrial arrhythmias were surprisingly seldom noted in the ECG's. Atrial premature beats were observed in 2 cases. There was no case of indubitable auricular flutter.

This diagnosis might however be discussed in case No. 32. There was in this case a massive, recent infarct in the lateral part of the right auricular appendage. Regular ventricular tachycardia without distinctly visible P-waves was present in 2 ECG's taken 9 and 8 weeks before death. No distinct P-waves were present, but they may be hidden in the ventricular complexes. The ventricular rates were 110 and 120/min. The available ECG's permit in this case no definite judgement.

Sinu-auricular block of the classical "dropped beat" type was not observed. The author finds this fact worth mentioning for two reasons. The first one is, that dropped beats are not uncommon in cases of ventricular infarct (observed in 12/372 cases in series C), suggesting an extension of the infarct to the atria. The second reason is, that infarcts were often found in the surroundings of the sinus node (though apparently respecting the node itself).

Some observations in case 152 may be discussed in this connection. There was in this case a recent infarct in the lateral wall of the right atrium and old infarcts in the auricular appendage. Nine ECG's were taken during 6 months before death. In some ECG's low P-waves were present, sometimes they were barely visible and in 2 ECG's (the last one taken 5 weeks before death) no P-waves at all could be observed. The ventricular rhythm was regular in all ECG's except for the presence of ventricular premature beats. The absence of P-waves in some of the ECG's may be explained in several ways — *inter alia* as the consequence of a sinu-auricular block ("auricular standstill"). In view of the extremely indistinct P-waves, which were registered in some

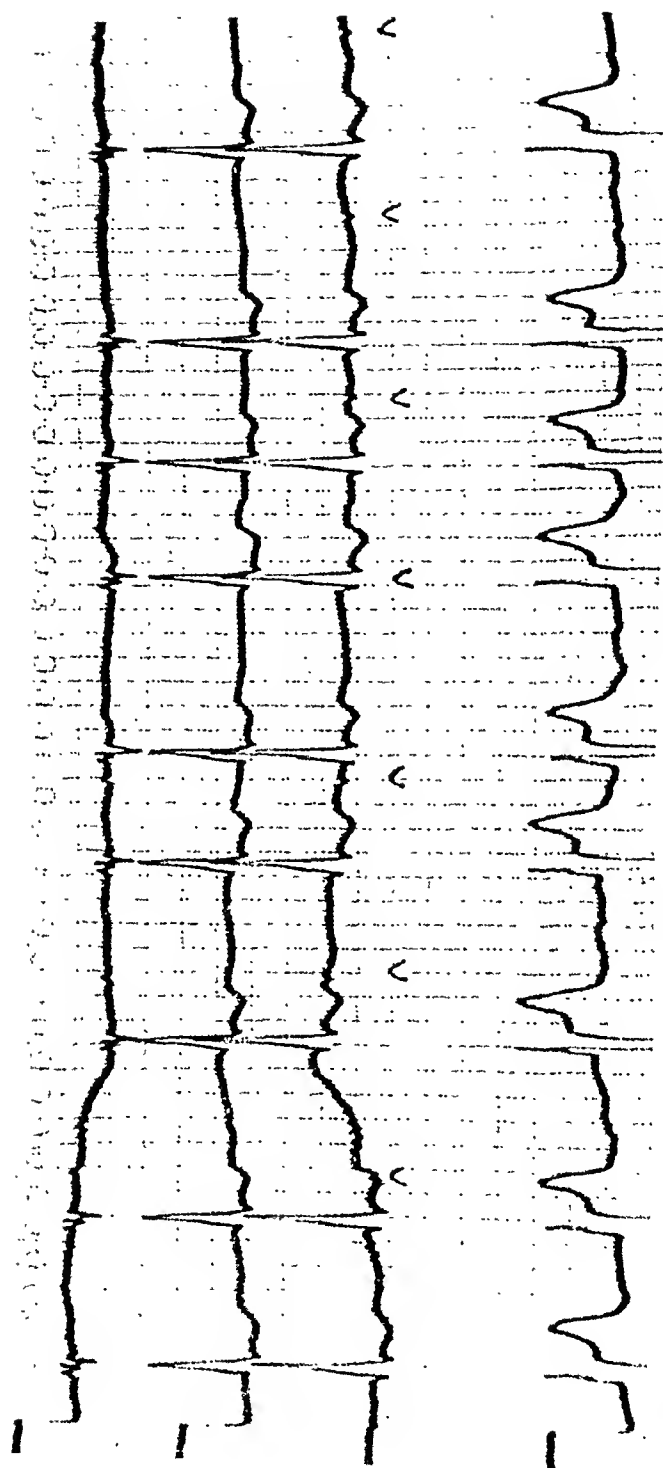


Fig. 29. Ser. A. case 27. Right atrial infarct. Arrhythmia perpetua, probably auricular fibrillation. A series of P-like deflections with regular intervals are marked with  $\wedge$ . Further comments on p. 83. ECG obtained 2 days before death.



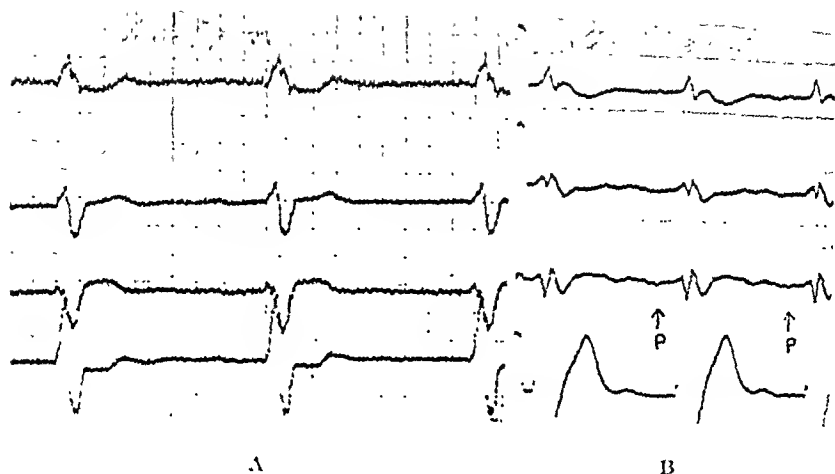


Fig. 30. Ser. A, case 152. Multiple infarcts of varying age in the right atrium. During 6 months before death ECG's without visible P-waves (A) had alternated with ECG's with barely visible P-waves (B).

ECG's (see fig. 30) the author is inclined to assume, that the "nearly" invisible P-waves present in some of these ECG's may in other ECG's simply be "quite" invisible. The case should then only be an illustration to the tendency for low P-waves in atrial infarcts discussed in the following section.

### *The P-waves.*

Striking abnormalities or actual changes in the contour of the P-waves were as a rule not observed. The P-waves were sometimes of normal amplitude but often definitely *low* in all leads. High P-waves of the type described by MASTER and other authors (p. 16) were at any rate not observed. It may be added, that high P-waves were seldom observed even in series C.

An observation regarding the P-waves in case No. 137 should be specially commented on.

This case was the only one of extensive *left* atrial infarct. Areas of infarct were found in the auricular appendage and in the lateral wall of the left atrium. There were in addition multiple infarcts in the ventricles, *inter alia* a recent one in the lateral wall of the left ventricles. Inveterate, occluding thrombi were found in the right coronary and in the interventricular branch of the left coronary and a recent thrombus was present in the initial segment of the circumflex branch of the left coronary.

This patient had during some years before death been treated several times in this hospital under the diagnosis acute myocardial infarction. There had always been distinct and pointed P-waves in lead I in numerous previous ECG's,

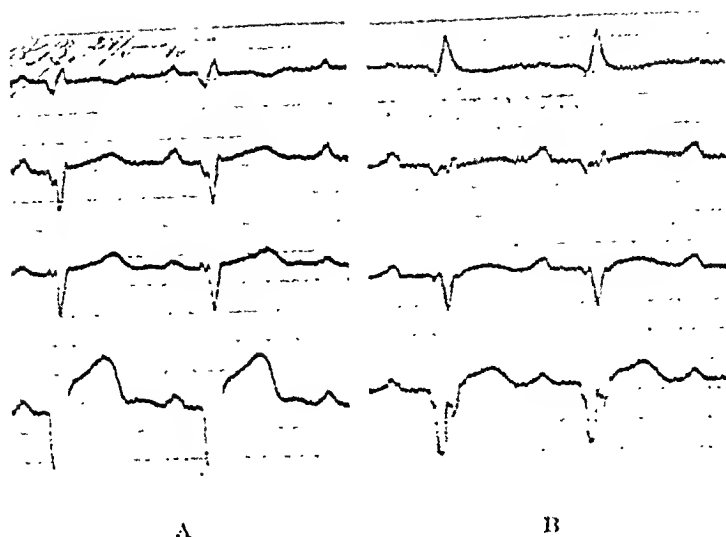


Fig. 31. Ser. A. case 137. Left atrial infarct. Earlier the P-waves in lead I were always marked (A). During the weeks before death they became indistinct (B).

including one taken 5 months before death, but in 2 ECG's taken during the month before death the P-waves of lead I were low and rather indistinct (fig. 31).

It has been mentioned (chapter IV) that the P-waves of lead I are regarded by some authors as representing mainly action potentials from the left atrium. A lowering ("loss of potentials") of the P-waves might theoretically well be due to atrial infarction and the finding in case 137 is consequently of some interest. It must however, be admitted, that the change in the contour of the P-waves may also have been brought about by other factors than the atrial infarct present.

#### *Duration of the P-Q (P-R) interval.*

Changes in the duration of the P-Q interval should theoretically reflect disturbances in the atrio-ventricular conduction mechanism. Both atrial and ventricular lesions may produce such disturbances and an attempt to differentiate the effects of atrial and ventricular lesions in cases of A-V block would be a very laborious enterprise of little practical interest. It may however be mentioned, that A-V block of different degree was present in 3 cases of atrial infarct (cases No. 166, 169 and 181). Infarcts were present in the dorsal walls both of the atria and the ventricles in all these cases. In another case (No. 152) was it difficult to decide if A-V block was present or not because of the indistinct P-waves.

*Abnormally short P-Q intervals* are, however, of a certain interest in this connection.

The present author described in a previous paper (1943) some cases of ventricular infarct with a short P-Q interval and positive P-waves. The author regarded a P-Q interval below 0.12 as certainly abnormal in the age groups in which coronary heart disease is common. In a material of 184 cases of myocardial (ventricular) infarct the author observed 10 cases with such a short P-Q interval. Some ECG's of this type are reproduced in fig. 32. Special observations in the ECG's from some of these cases made the author suspect that the short P-Q interval might reflect an extension of the infarction to atrial structures.

As a matter of fact, the search for an anatomical proof of this hypothesis was the starting point from which this study developed. The hypothesis was based on the following theoretical considerations.

Short P-Q intervals may simply indicate a quicker passage than usual of the impulse from the atria to the ventricles. This is probably the case in the WPW syndrome (p. 17). In other cases must it be assumed that the reduction of the P-Q interval is due to a *relative* retardation of the activation of the atria, *relative* as compared with the activation of the ventricles.

Nodal rhythm represents the classical example of such a condition. If the nodal impulse center is situated not too far from the atrial extremity of the A-V system, then the P-waves may appear before the ventricular complexes and a short P-Q result. Nodal rhythm occurs when there is some hindrance for the passage of the sinus impulse to the A-V node, or if the rate of the sinus automatism is, for some reason, slower than the inherent rate of the nodal automatism. Atrial infarcts could theoretically cause both conditions.

It is accepted in most textbooks, that in nodal rhythm the P-waves are usually negative. According to ROTHBERGER & SCHERF this feature is, however, not invariable.

In the cases described by the present author, the P-waves were positive, or at least not negative in most leads. Nodal rhythm seemed for that reason not to represent a satisfactory explanation of the short P-Q interval. The picture was definitely not that of the WPW syndrome (no  $\Delta$ -wave). It seemed improbable that *acceleration* of the passage of the impulse through the A-V system could be caused by myocardial infarction. Another explanation was however possible.

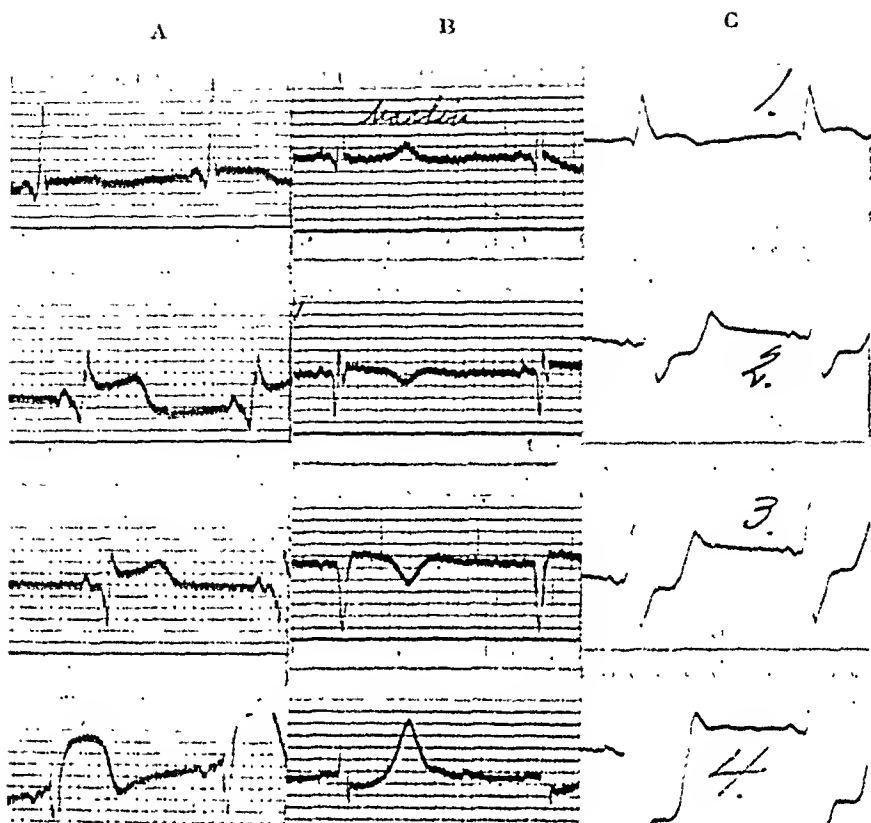


Fig. 32. Other examples of short P-Q intervals in cases of ventricular infarcts from series C.

- A. Sahlgrenska sjukhuset, Medical department I, hosp. record 2404/1941. Autopsy: recent infarcts in ventral and dorsal walls of both ventricles. No mention of the atria in the routine autopsy protocol.
- B. Sahlgrenska sjukhuset, medical department I, hosp. record 1919/1943. This patient survived.
- C. Akademiska sjukhuset, medical clinic, hosp. record 584/1937. Autopsy: large, recent infarct of the ventral wall of the left ventricle. Mural thrombosis of the left atrium.

In 2 of these cases the author observed atrial premature beats of a definitely unusual type. In one case they were followed by an *exact compensatory pause*, in the other case they were actually *interpolated*. To judge from its undisturbed activity the sinus node had obviously not been reached by the impulse of the premature beats. These observations made the author assume the presence of a zone of blocking (B in fig. 33), preventing the extrasystolic impulse (assumed to arise in the main part of the atrium) from reaching the sinus node. The same zone of blocking could also be assumed to retard the passage of the normal sinus impulse to the main part of the atrium. If there was no obstacle for the passage of the sinus

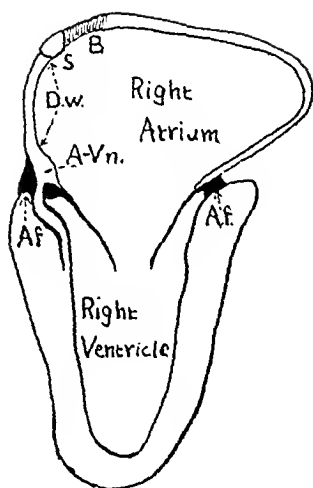


Fig. 33. Diagram to the theory of short P-Q interval caused by an intra-atrial zone of blocking. Af annulus fibrosus, A-Vn A-V node, B zone of blocking, Dw dorsal wall of the right atrium, S sinus node.

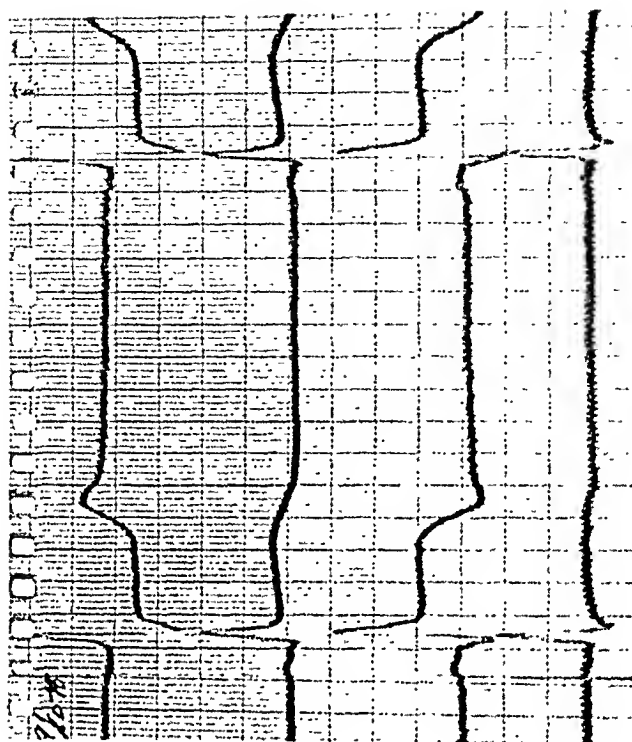
impulse to the A-V system (through the dorsal wall — D. W. in fig. 33) then the zone of blocking would account also for the short P-Q interval. The mechanism assumed is similar to that of short P-Q in nodal rhythm: a delayed activation of the main part of the atrial myocardium, coinciding with a normal conduction time from the sinus node to the ventricles.

Similar effects of intra-atrial conduction disturbances had earlier been noted in animal experiments by ROTHBERGER & SCHERF and by CONDORELLI (1929). SPUEHLER (1939) was the first to suggest this mechanism to explain short P-Q intervals observed clinically.

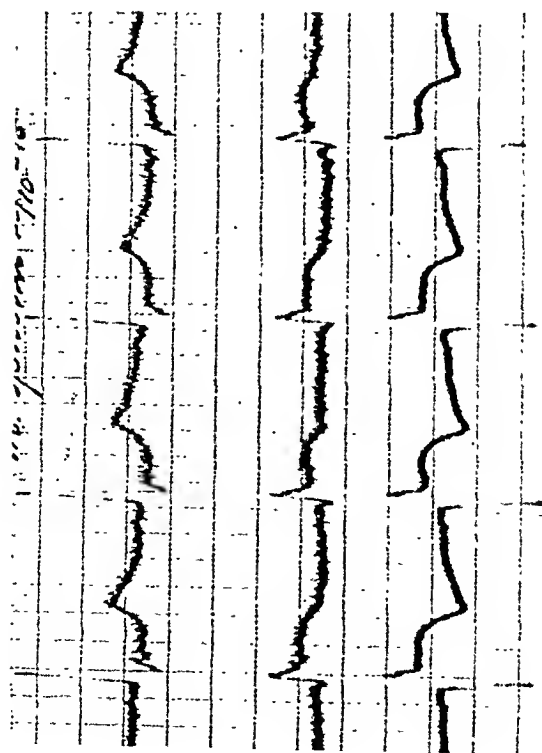
The present author suspected, that the zone of blocking assumed to be present in the cases mentioned might be a myocardial infarct in the right atrium. Theoretically, such an infarct should include a considerable part of crista terminalis, along the ventral margin of the sinus node, but leave some part of the dorsal wall undamaged to permit the passage of the sinus impulse to the A-V node.

There is only one case with a short P-Q interval in series A, but the anatomical findings in this case were very instructive.

The case was a diabetic woman who was 51 years old when she had a typical, acute ventricular infarct of the T<sub>III</sub> type. There had been no other conspicuous signs of coronary disease in her past history and she survived the infarct for 12 months before she died from crural gangrene, probably caused by a local arterial thrombosis. During the period of the acute infarct the P-Q time was 0.10—0.11 sec. and in the last ECG, taken 3 weeks before death, 0.12 sec. (fig. 34). Unfortunately no ECG from the time before infarction is



B



A

Fig. 34 Short P-Q intervals in a case of atrial infarct (Ser. A caso 183). Discussion on p. 90.

available. The P-waves were during the period of the acute infarct rather indistinct. In the first ECG, taken about 24 hours after the begin of the *status anginosus* they were negative in lead I but positive in leads II, III and IV (fig. 34 A), but in an ECG taken 3 days later (fig. 34 B) the P-waves were positive in lead I and remained so in numerous ECG's from the following time. The transitory occurrence of negative P-waves might justify the assumption of a simple nodal rhythm in this case. They were however negative only in lead I and the fact that they soon became positive (without any change in the duration of the P—Q-interval) made the author assume that the case was analogous to the other cases of myocardial infarct and short P—Q interval which have been discussed in this section.

Though the infarct was thus 12 months old at the time of death, the autopsy findings were unusually simple and plain. In general there was only moderate coronary atheromatosis, but a distinct stenosis of the right coronary (old thrombus) and a large scar in the dorsal part of the ventricular septum. The remaining ventricular myocardium was fairly normal. Nothing abnormal could be seen in the atria with the naked eye except for pericardial fibrosis, but under the microscope old organized thrombi were found in the right atrium. Numerous old infarct scars were found disseminated in the trabeculated part of the right atrium. The myocardium of crista terminalis was transformed into a solid fibrous scar from the level of "antrum" and at least 3—4 cm laterally. The sinus node was not destroyed and the structure of its medial part was fairly normal; a certainly pathological scarring was observed in its lateral part. The massive scar in crista terminalis was situated between the sinus node and the myocardium of the main part of the atrium, but in the structures uniting the sinus node with the A—V system (Torus Loweri and the dorsal part of the septum atriorum) only small scars were found.

The anatomical findings in this case thus fit in rather well with theoretical assumptions. The rarity of massive infarcts in the *crista terminalis* makes the coincidence particularly striking. It must however be admitted that the *anatomical* findings do not rule out the possibility of a nodal rhythm. Still another possible explanation for the short P-Q in case 183 will be discussed in the next section of this chapter.

The findings do at any rate constitute definite evidence for the author's assumption that an abnormally short P-Q in cases of "coronary" heart disease may indicate the presence of myocardial infarct in the right atrium. It is of a certain importance not to mistake this type of short P-Q in "coronary" heart disease for the short P-Q of the WPW syndrome, a syndrome that may sometimes present a clinical picture closely similar to that of acute myocardial infarction.

### *Ta-wave and P-Ta level.*

Displacements of the P-Ta levels were not common findings. A *depression* of the P-Ta's — to be regarded as significant in accordance with the discussion on p. 36 — was never observed in the cases of atrial infarcts. The author searched for depressed P-Ta's in the series B and C also but a really striking and definitely significant depression was only observed in 2 cases with right atrial thrombi and 2 cases with ventricular infarcts. They were all found in leads II—III. Naturally, the author cannot say whether atrial infarcts may not have been present in some of these cases; however the low incidence justifies the assumption that a depression of the P-Ta level is not a common consequence of atrial infarcts.

*Elevated P-Ta levels* were, on the other hand, present in some cases of atrial infarct.

An elevation of the P-Ta level in leads II and III is evident in an ECG from case 166 (fig. 35 A), which was taken 2 days before death. The condition is made especially evident by the presence in this case of a 2/1 A-V block. The P-Ta has the character of a long slope, that may be traced to at least 0.30 sec. from the beginning of the P-waves, a figure of the same magnitude as the normal P-Ta interval (chapter IV). The same sloping P-Ta's were found in an ECG taken the day before death. At autopsy a recent, occluding thrombus was found in the right coronary near to its origin, there was an extensive "posterior wall" infarct of the left ventricle and a not very extensive, recent infarct in the dorsal wall of the right atrium, confined to the smooth-walled "sinus"-part.

Similar, but less pronounced elevations of the P-Ta levels, at least in lead III were observed in 3 additional cases (ser. A case 169, 181 and 196). They were all cases of recent "dorsal" infarcts of the right atrium, combined with recent "posterior wall" infarcts of the ventricles. All died within 4 days after the onset of the infarct. Case 169 (fig. 36 A) had a recent thrombus near the origin of the right coronary and developed a 2/1 A-V block the day before death. In case 181 (fig. 35 C) a stenosis of the right coronary was the only finding which might explain the infarctions. Auricular fibrillation developed the day before death. Case 196 had a recent thrombus, occluding the right coronary 1 cm from its origin. Neither A-V block nor auricular fibrillation were recorded before death, but there was an elevation of the P-Ta in lead III amounting to 0.5 mm.



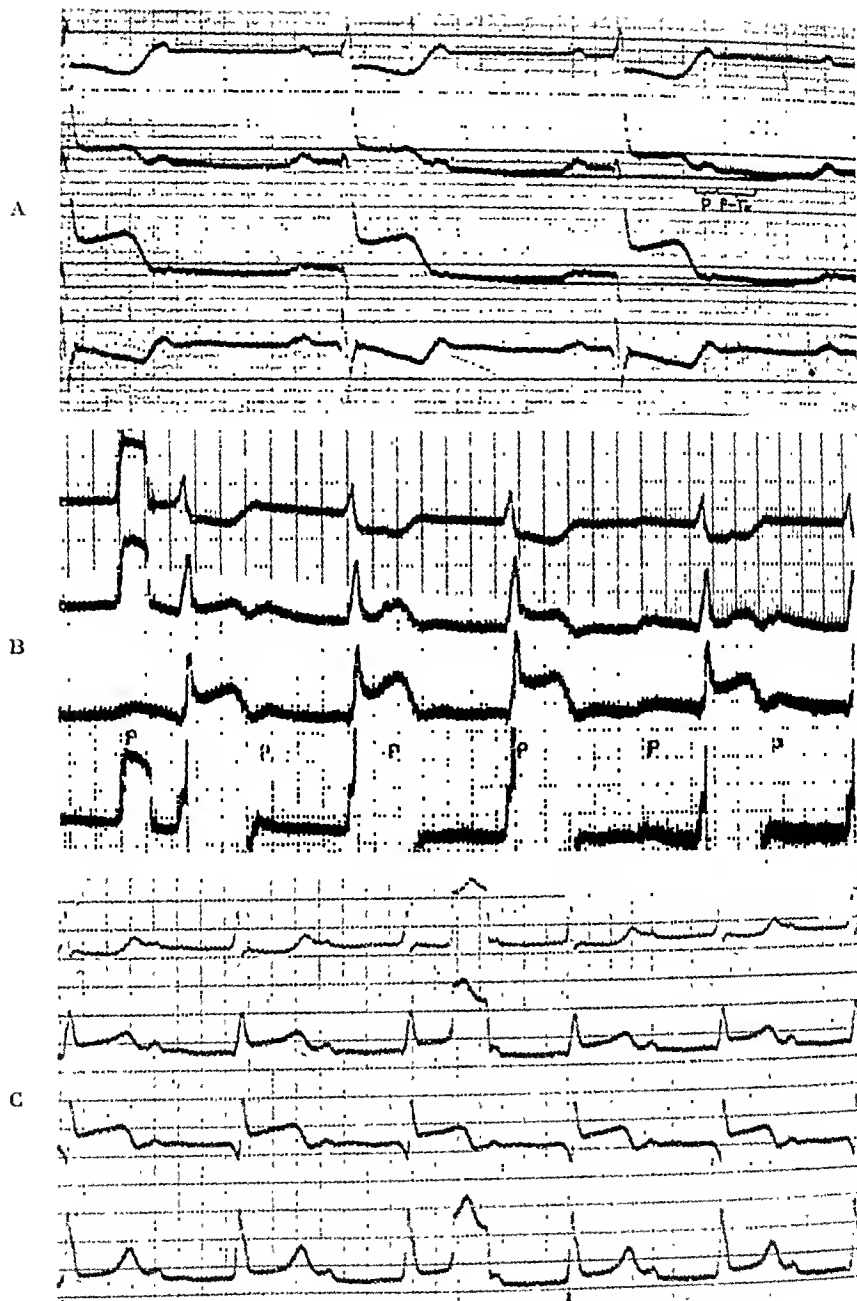


Fig. 35. Elevated P-Ta levels in leads II—III.

- A. Ser. A case 166. Recent infarct in the dorsal wall of the right atrium.  
 B. Similar ECG from a case of posterior wall infarct in series C (Sabbatsbergs sjukhus, medical department II, hosp. record 562/1942). No reference to the atria in the routine autopsy protocol.  
 C. Ser. A case 181. Recent infarct in the dorsal wall of the right atrium.

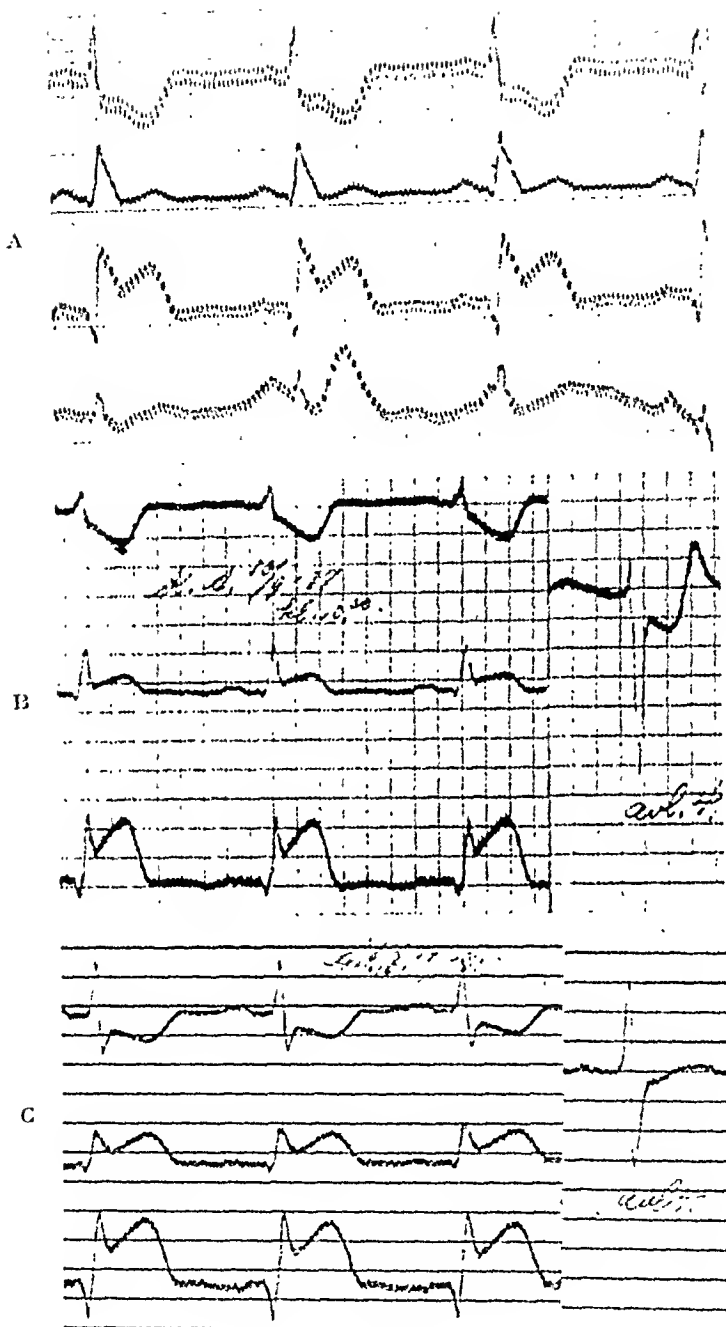


Fig. 36. Slightly elevated P-Ta levels in lead III.

A. Ser. A case 169. Recent infarct in the dorsal wall of the right atrium.

B. and C. Similar findings in cases with posterior wall infarction from series C (Akademiska sjukhuset, medical clinic, hosp. records 1866/1938 and 99/1938).

Autopsy to B: large, posterior wall infarct, thrombus near the origin of the right coronary, no mention of the atria.

Autopsy to C: no coronary thrombosis, otherwise as B.

At first the author regarded the findings in case 166 as quite exceptional. However, on searching through the cases of series C the author found another 5 cases with a corresponding elevation of the P-Ta levels in lead III (sometimes also in lead II). All these cases also had recent ventricular infarcts of the  $T_{III}$  type (figs. 35 B, 36 B and 36 C). All cases died some days after the onset of the infarct, and the autopsies invariably showed extensive "posterior wall" infarcts and (in all but one case) recent occluding thrombi near the origin of the right coronary. Information regarding possible atrial lesions could not be obtained from the autopsy reports.

It may finally be mentioned, that the same picture is present in an ECG representing "posterior wall infarct and complete auriculo-ventricular block" reproduced on p. 229 in the textbook on Cardiovascular Disease by SCHERF and BOYD (1947).

The present author has never seen elevated P-Ta levels in leads II—III in cases other than the ones just mentioned. Most of them had a recent thrombus near the origin of the right coronary, "posterior wall" infarcts were present in all cases and in 4 cases infarction could actually be demonstrated in the dorsal wall of the right atrium as well. The picture is sufficiently uniform to justify the conclusion, that an *elevation of the P-Ta level in lead III (and lead II) is a sign of infarction in the dorsal wall of the right atrium.*

The ECG picture in fig. 36 C suggests another explanation of the "short P-Q in coronary disease" than the one proposed on p. 89. It is possible that some such cases represent the combination of very low P-waves and elevated P-Ta's in leads II—III. The elevated P-Tas could be mistaken for P-waves and the P-Q would consequently seem abnormally short.

There is an obvious analogy between the ECG findings in the dorsal infarcts of the right atrium and the  $T_{III}$  type of ECG in "posterior wall" infarcts of the ventricles. It was tempting to try to extend the analogy and search for an elevation in lead I or in chest leads in the cases with a ventral distribution of right atrial infarcts. Such elevations were noted in 2 cases (ser. A case 113 and 132) but a closer scrutiny revealed that they were only apparent ones and due to the presence in lead I of a negative U-wave (see p. 37). Similar ECGs were found in 5 cases in series C, but negative U-waves proved to be the probable explanation in these cases also. An atrial analogy to the ECG picture of the  $T_I$  type of ventricular infarcts was thus not observed.

## Summary.

It has not been possible to deduce from this material any *general* clinical signs, which might be regarded as typical of atrial infarction.

*Anginal pain* proved to be no common or important sign of atrial infarction.

*Auricular fibrillation* was present in about half the cases of atrial infarction. In many cases had it been present before the onset of infarction. Auricular fibrillation *developed* in about 1/3 of the cases which had regular rhythm before the infarction. On the other hand, auricular fibrillation developed, under comparable conditions, also in many cases without atrial infarcts. It is thus at any rate a sign of too little specificity to be of any value for the clinical diagnosis of atrial infarction.

Other atrial arrhythmias were seldom observed and without any interest for diagnosis.

Some observations were made in the *atrial part of the ECG* representing more or less reliable signs of atrial infarction.

*Elevated P-Ta levels in leads II and III* were observed in some cases with infarcts in the *dorsal wall of the right atrium*. There is reason to assume that this ECG picture is diagnostic for dorsal infarcts of the right atrium.

There is reason to assume, that the ECG picture of abnormally short P-Q intervals and positive P-wave may be a sign of infarction in the right atrium.

The P-waves were sometimes of normal form and amplitude, but in many cases they were *low* or even indistinct. Conspicuous changes in the contour of the P-waves were seldom observed in this material. *Certain* conclusions of general interest for the diagnosis of atrial infarction could not be derived from the appearance of the P-waves.

## Addendum.

In a paper, which appeared during the printing of this work, H. HELLERSTEIN (Am. Heart J. 36: 422, 1948) has described a case of infarct in the dorsal walls of both atria. HELLERSTEIN observed in this case elevations of the P-Ta's in leads II and III quite similar to the ones described in the authors case 166 (p. 93).

## General Summary.

The original purpose of this investigation was that of studying *myocardial infarcts in the atria of the heart*, starting from cases in which the diagnosis had been made at autopsy. Atrial mural thrombi were used as indicators for the tracing of atrial infarcts. The author's main material thus comprises a series of 192 autopsy cases with atrial thrombi, some of which had arisen on the basis of atrial infarcts. As a consequence of this method of sampling the *etiology of atrial thrombosis* has also become a central topic of the present study.

The main results and viewpoints which have been accounted for in this study may be summarized as follows.

The reasons for the high incidence of myocardial infarcts in the left ventricle are discussed in CHAPTER I. The author ventures to suggest that infarcts in other parts of the heart are often overlooked.

A review of earlier literature on atrial infarction is given in CHAPTER II. It may briefly be stated here, that most authors regard atrial infarcts as exceptional. Very little is known about the clinical picture of such lesions.

A short account for the authors material and methods is given in CHAPTER III.

Some pertinent data regarding the anatomy and physiology of the atria are given in CHAPTER IV. The author should like to stress the following statements, which are little known and important for the main scope of this study.

1. The trabeculated part of the atrial wall occupies a much larger part of the inner surface in the right than in the left atrium.
2. The author describes a complicated system of contiguous recesses (auricular sinus) as typical of the *right* atrium. The auricular sinus play an important rôle in the development of thrombi in this chamber of the heart.
3. Numerous possibilities for extra-coronary nutrition of the thin atrial walls make the atrial myocardium less dependent on the coronary blood supply than the ventricular myocardium.

The etiology of atrial mural thrombosis is discussed in CHAPTER V. The discussion is based on morphological (gross and histological) observations in the main material (*series A*, 192 cases with atrial

thrombi) and, in addition, on a statistical analysis of a material of 382 cases of atrial thrombosis, registered during the years 1936—1945 at the University Institute of Pathology in Uppsala (series B).

The author concludes from a preliminary discussion that stagnation factors and wall lesions may both be of importance for the formation of atrial thrombi. However, the importance of stagnation factors is easily overestimated and there is reason to believe that lesions of the atrial wall may be decisive in the formation of most atrial thrombi. Little is known, however, about the types and the frequency of such wall lesions. The following observations were made in the author's materials.

*Left atrial thrombi* were especially common in cases with rheumatic heart disease. Two fairly well-defined types of left atrial thrombi could be distinguished. *Recess thrombi* were usually rounded, simple clots occurring in the left auricular appendage. *Surface thrombi* were broadly fixed to the endocardium of the smooth-walled part of the atrium and had a peculiar general structure (characterized by the term "callous thrombi"). A *mural endocarditis* (probably a manifestation of rheumatic heart disease) was found beneath most *surface thrombi*. Conspicuous wall lesions were seldom found beneath *recess thrombi*.

*Right atrial thrombi* were somewhat commoner than left ones. They were particularly common in cases with coronary heart disease and were usually not differentiated into types corresponding to those of left atrial thrombi. The characteristic appearance of extensive right atrial thrombi (fungiform thrombi) is probably mainly due to the presence of the auricular sinus. *Atrial infarcts* or less extensive "coronary" myocardial lesions were found beneath most *right atrial thrombi*.

Conspicuous lesions in the atrial wall were thus observed beneath the thrombi in about the half of all atrial mural thrombi examined. It is very difficult to ascertain the importance of *stagnation* factors for their formation. The author attempted to analyse in series B the importance of *auricular fibrillation*, which is generally said to be related to atrial thrombosis, presumably acting as a stagnation factor. There was in series B generally a significant positive correlation between auricular fibrillation and atrial thrombosis. This statement is, however, with certainty valid only for left atrial and bilateral thrombi. Most cases with right atrial thrombi only had regular

sinus rhythm during life. The author concludes finally, that the positive correlation between auricular fibrillation and atrial thrombosis is probably not due only to the mechanical, (stagnation) effect of auricular fibrillation.

The mural endocarditis observed beneath surface thrombi is briefly described in CHAPTER VI.

In CHAPTER VII a special study is devoted to the histological picture of atrial infarction. It is pointed out that an area of myocardial infarction usually consists of 2 zones representing different degrees of myocardial damage, a *central zone* of complete ("hyaline") necrosis and a *marginal zone* in which the myocardial degeneration is less advanced. The marginal zone is particularly conspicuous in the area immediately beneath the endocardium (the lumen zone) in which the myocardium may be nourished from the blood in the lumen of the heart. The myocardium in the marginal zone often consists of more or less "empty" fibres, poor in myofibrils; the author has called this type of degeneration foamy degeneration.

The central zone dominates the histological picture of ventricular infarct. In the thin wall of the atria most parts of an infarcted area belong to the marginal zones (lumen zone and pericardial zone), presenting signs of a less advanced myocardial damage. Atrial infarcts may thus be difficult to recognize even under the microscope. The "coronary" etiology of such lesions may however be proved by the demonstration of a distinct differentiation into lumen zone and central zone.

The author measured the breadth of the lumen zone in 29 cases of right atrial infarction and in 16 cases of left ventricular infarction. The mean breadth of the right atrial lumen zone was  $0.13 \pm 0.007$  mm and of the left ventricular lumen zone  $0.23 \pm 0.015$  mm.

The predominant histological picture in atrial infarction was that of foamy degeneration. The author has demonstrated that fibres presenting this typical appearance (in cases of coronary heart disease) are regularly *rich in glycogen*. The author suggests, that some cases which have been described in the literature under the diagnosis *cardiomegalia glycogenica circumscripta* represent coronary degeneration zones of this type.

A general morphological analysis of the author's material of atrial infarct cases is to be found in CHAPTER VIII. In series A 46 cases of atrial infarct were observed. There was in addition 33 cases of

less extensive atrial lesions of a "coronary" type (labelled "minor lesions"). The author observed only one case of atrial infarct without mural thrombosis.

In 46 cases the infarcts were observed in the right atrium and in one case only in the left atrium. The author is inclined to regard the high oxygen saturation in the blood of the left atrial lumen as the main cause for the rarity of left atrial infarcts.

The author found reason for distinguishing 2 main distribution types of right atrial infarcts: a *dorsal type*, occurring in the dorsal wall of the right atrium and regularly coinciding with "posterior wall" infarcts of the ventricles and a *ventral type* occurring in the auricular appendage and adjacent parts of the atrial wall (antrum, lateral wall). In most cases of *ventral* infarcts conspicuous ventricular infarcts of the same age had not been recorded.

According to the authors experience atrial infarcts are no very rare lesions. They are to be expected in *at least* 1 per cent of the cases of an average autopsy material.

It is possible that atrial infarcts are commoner during life but that cases with such lesions usually survive. Atrial infarcts are as a rule no extensive lesions and are not likely to affect seriously the general state of the patients.

This assumption may furnish an explanation of the meagre results of the authors search for a typical clinical syndrome of atrial infarction, accounted for in CHAPTER IX. All the author's cases died of some other disease and no *general* clinical symptoms, with certainty attributable to the atrial infarcts, could be discerned in their terminal syndromes. In some cases described in the literature the atrial infarct became fatal as a consequence of a rupture of the atrial wall, this consequence did not occur in the author's material. The following observations may, however, be mentioned.

*Anginal pain* is at any rate no important symptom of atrial infarction.

Auricular fibrillation *developed* somewhat more often in cases with atrial infarcts than in cases with right atrial thrombi without infarcts. The difference was, however, not statistically significant and the appearance of auricular fibrillation must be said to be a sign of little practical value for the diagnosis of atrial infarction during life. Other atrial arrhythmias were observed only in a few cases.



The P-waves were in some cases normal, in other cases low or even indistinct. A very marked lowering of the P-waves in lead I was observed in the only case of left atrial infarction present in this series.

The author assumed in an earlier paper, that abnormally short P—Q intervals, observed in some cases of myocardial infarction, might be a sign of atrial infarction. The post mortem findings in one of the present cases were in favour of this hypothesis.

A significant depression of the P—Ta levels was not observed in this material but there is reason to assume that *elevated P—Ta-levels in leads II and III are diagnostic for infarcts in the dorsal wall of the right atrium.*

Table 6. Distribution of the atrial thrombi of series A with regard to the site of the thrombi and the main diagnosis.

	VMI	Cscl+ Hpt	Hpt	R	O
R.....	29	30	3	10	24
R+L.....	7	9	2	17	9
L.....	7	—	1	33	11
Total	43	39	6	60	44

For explanation of the abbreviations, see table 2.

Table 7. Distribution of the cases of series A with regard to the site of the thrombi and the heart rhythm before death.

	S	SF	F
R.....	55	12	28
R+L.....	7	6	29
L.....	8	3	40
Total	70	21	97

S = regular sinus rhythm.

SF = auricular fibrillation had developed within 2 months before death.

F = auricular fibrillation had been present for more than 2 months before death.  
Cases with thrombi in the right atrium = R, in both atria = R+L, in the left atrium = L.

## Explanations.

*Case No.* The first figure given is the number of the case in the author's index of series A.

MU = medical clinic, Akademiska sjukhuset, Uppsala.

KU = surgical clinic, Akademiska sjukhuset, Uppsala.

SbMII = medical department (II), Sabbatsbergs sjukhus, Stockholm.

SG = medical department, St Görans sjukhus, Stockholm.

SSMI, SSMII, SSKII = medical departments (I and II) and surgical department (II), Sahlgrenska sjukhuset, Gothenburg.

Vasä = Vasa sjukhus, Gothenburg.

*Age, sex.* M. = male, F = female.

*Hpt* = arterial hypertension (+ = present, — = not present).

*Coron. scler.* = coronary arterial sclerosis (+ + = extensive, + = moderate). Thrombosis of the right coronary = D, of the interventricular branch of the left coronary = S, of the circumflex branch of the left coronary = C.

*Ventr. infarct* = ventricular infarct (A = anterior wall, P = posterior wall, L = lateral infarct; r = recent, v = old.)

*A. P.* = Anginal pangs (+ = registered, — = not registered).

*Congest. heart fail.* = congestive heart failure (+ = signs of congestive heart failure had been prominent for more than 2 months before death. — = other cases).

*Site of atrial infarct.* The figures refer to the four regions of the right atrium mentioned on p. 75. I = auricular appendage, II = cranial part of crista terminalis with "antrum" atrii dextri, III = lateral wall, IV = dorsal wall and dorsal part of the septum.

If these figures are given in the table, then myocardial infarcts had been observed in sections available from the corresponding regions. "O" in the place of a roman figure means that there is no infarct in available sections from this region. "?" means that no sections are available from the region in question.

*Inf. age* = age of atrial infarct. V = old, R = recent.

*Heart rhythm:* S = regular (sinus?) rhythm, F = chronic auricular fibrillation, S/F = fibrillation had developed during the 2 last months before death, (F) = uncertain whether the auricular fibrillation registered was chronic or acute.

*Electrocardiograms:* only ECG's from the last two months before death (= a. m.) are referred to. — = no ECG's available.

Table 8. Cases with atrial

Case No.	Age Sex	Diagnosis	Hpt	Coron. scler.	Ventr. infarct.
6. SSMII HR 58/44	58 F	Hypertensive heart disease Pulmonary arterial sclerosis	+	+	—
20. Vasa HR 766/44	82 F	Coronary arterial sclerosis Inveterate myocardial infarct.	+	++	A. v.
27. Vasa HR 886/44	62 F	Hypertensive heart disease Coronary arterial sclerosis Inveterate myocardi. infarct.	+	+	P. v.
30. Vasa HR 558/44	82 M	Hypertensive heart disease Coron. art. atheromatosis Cerebral softening	+	+	P. v.
32. SSMI HR 2132/44	39 F	Chronic myocarditis Pulmonary embolias	—	—	P. v.?
40. SSMI HR 2091/44	69 F	Myocardial infarction	+	++ D	P. r.
46. MU HR 1736/44	60 F	Coronary art. scleros. Ruptured myoc. infarct (ventr.) Pulmonary embolias	+	++ SD	A. r. P. r.
47. MU HR 1881/44	73 M	Coronary art. sclerosis Multiple pulm. and system. embolias	—?	+	P. v.
58. SG HR 571/47	73 F	Hypertensive heart disease Coronary art. sclerosis	+	+	—
72. Vasa HR 766/45	73 M	Coronary art. sclerosis Old myocardial infarct Hypertensive heart disease	+	++	P. v.
83. MU HR 1224/45	53 M	Tuberc. spondylitis Grave kyphoscoliosis <i>Cardiopathia kyphoscoliotica</i>	+	—	—
94. MU HR 546/46	74 F	Myeloma Coronary art. sclerosis Bronchopneumoniae	—	+	—
95. MU HR 402/48	68 M	Coronary art. scleros. Inf. myoc. (inveterate) with rupt. of interventr. sept.	—	++	A. v.
103. MU HR 109/46	74 M	Coronary art. sclerosis Chronic bronchitis, bronchi- ectasiao	—	++	P. v.

## infarction in series A.

A. P.	Cong. heart fail.	Site of atrial infarct	Inf. age.	Heart rhythm.	Electrocardiogram
?	+	I. II. ? ?	V	S	—
—	+	I. II. ? ?	VR	F	—
—	+	I. ? III. ?	VR	F	one, 2 days a. m. (see p. 85).
(+)	+	I. I. O. O.	R	S	one, 2 days a. m. Low P-waves.
—	+	I. O. III. ?	R	S/F	one, 2 months a. m. (p. 84) Aur. fibrill. diagnosed clinically before death.
+	—	I. II. III. IV.	R	S	—
—	+	I. II. ? ?	R	S	one, 9 days a. m. P-waves pos. in lead I, absent in lead II, neg. in lead III.
—	+	I. O. ? ?	R	F	—
—	+	I. II. O. O.	VR	(F)	one, 53 days a. m., auricular fibrillation.
—	+	I. II. O. ?	R	S/F	two ECG's, one 15 days a. m. sinus rhythm, atr. ECG norm. one 6 days a. m.: aur. fibr.
—	+	I. II. ? ?	R	S	one, 3 days a. m. Atrial ECG without striking alterations.
—	+	I. ? ? ?	V	F	one, 13 days a. m. aur. fibrill.
+	+	I. II. O. O.	VR	S	Five ECG's from the month a. m. Nothing abnormal in the atrial part of the ECG.
+	+	O. II. O. IV.	VR	S/F	One ECG 6 weeks a. m. sinus rhythm. Atrial ECG normal Aur. fibr. 1 month a. m. (2 ECG's).

Case No.	Age Sex	Diagnosis	Hpt	Coron. scler.	Ventr. infarct.
104. MU HR 1968/45	67 F	Hypertensive heart disease Coronary art. sclerosis Cerebral art. thrombosis	+	+	—
110. SG HR 351/46	60 F	Coronary art. sclerosis Recent myocardial infarction	—?	++ D	P. r.
112. SG HR 1148/46	76 F	Moderate mitr. and aort. stenosis. Coronary art. sclerosis	—	++	(P. v.?)
113. SG HR 573/45	72 F	Hypertensive heart disease Coronary art. sclerosis Recent myocardial infarction	+	++ D	P. r.
116. MU HR 1962/46	55 F	Hypertensive heart disease Coron. art. scler. Moderate mitr. and aort. sten. Pericarditis sicca	+	+	—
121. MU HR 2297/46	56 F	Coronary art. sclerosis Acute, purulent peritonitis	—	++	—
123. MU HR 2252/46	70 M	Mitral and aortic stenosis Coronary art. sclerosis	—	+	—
127. SbMII HR 591/45	60 F	Coronary art. sclerosis Hypertensive heart disease	+	++	A. v.
129. SbMII HR 506/46	72 M	Hypertensive heart disease? Coronary art. embolism	+	(+)	(A. v.)
130. MU HR 2455/46	53 M	Hypertensive heart disease Uremia. Multiple pulm. art. embolias	+	(+)	(A. v.)
132. MU HR 1604/46	73 M	Coronary art. sclerosis Recent myocardial infarction Hypertensive heart disease	+	++	A. v. F. r.
133. MU HR 1142/46	63 M	Aortic stenosis (extreme fibrosis of the papillary muscles)	—	—	A. v.
137. MU HR 2850/46	73 M	Hypertensive heart disease Coronary art. sclerosis Recent myocardi. infarction	+	++ C	A. v. P. r. L. r.
139. SG HR 1236/46	58 F	Coronary art. sclerosis Recent myoc. infarction Hypertensive heart disease	+	++ D	P. r.

A. P.	Cong. heart fail.	Site of atrial infarct.	Inf. age.	Heart rhythm.	Electrocardiogram
?	+	I. II. O. O.	VR	F	One ECG 1 month a. m. auricular fibrillation.
+	—	I. II. III. O.	VR	S	—
—	+	I. II. ?. ?.	V	F	—
+	—	I. O. III. IV.	R	S	One ECG 6 days a. m., low P-waves. Apparently elevated P-Ta <sub>I</sub> (Neg. U-waves!).
(+)	+	I. II. O. O.	R	S	Two ECG's from the 2 last months. Nothing abnormal with the atrial part of the ECG.
—	+	I. II. III. O.	VR	S/F	Regular rhythm (no ECG) 2 months a. m. ECG 3 weeks a. m. auricular fibrillation.
—	+	I. II. ?. ?.	R	(F)	—
+	+	I. II. O. ?.	V	F	—
?	+	I. II. O. ?.	V	F	—
—	+	I. O. III. O.	R	S/F	One ECG 1 month a. m. Nothing abnorm. in atr. part of ECG. Six days a. m. aur. fibr. (2 ECG's).
+	+	I. II. III. IV.	R	S/F	Sinus rhythm 1 month a. m. Aur. fibr. in 2 ECG's from 6 days and 3 days a. m.
+	+	I. II. O. O.	VR	S/F	Two ECG'S 8 and 1 days a. m. Sinus rhythm with normal atrial parts in both ECG's short paroxysms of aur. fibr.
+	+	left atr.	R	S	Two ECG's from the last month a. m. P <sub>I</sub> disappear. (See p. 86.)
+	+	O. O. III. IV.	R	S	One ECG 7 days a. m. Nothing abnormal in atrial part of ECG.

Case No.	Age Sex	Diagnosis	Hpt	Coron. scler.	Ventr. infarct.
144. MU HR 2542/46	64 F	Chronic endocarditis with a grave aortic stenosis	+	(+)	—
148. Vasa HR 1050/46	59 M	Coronary art. sclerosis Old myoc. infarcts Hypertensive heart disease	+	++	A. v. P. v.
152. MU HR 3382/46	73 F	Coronary art. sclerosis Hypertensive heart disease	+	++	—
153. SSKII HR 5/47	71 M	Coronary art. sclerosis Myocard. infarction Prostatic hypertrophy.	?	++ D	P. r.
158. MU HR 3290/46	76 F	Coronary art. sclerosis	?	++	P. v.
164. MU HR 373/47	77 F	Chronic and acute endocarditis with aortic stenosis Abscessus pulm dx	—	++	—
166. MU HR 810/47	49 M	Coronary art. sclerosis Recent myocard. infarction	?	++ D	P. r.
169. MU HR 935/47	67 M	Coronary art. sclerosis Recent myocardial infarction	?	++ D	P. r.
170. KU HR 105/47	72 F	Hypertens. heart disease. Diabetes mellitus Gangraena podis dx (arterial embolia)	+	+	—
181. MU HR 2159/47	65 F	Diabetes mellitus. Coronary art. sclerosis. Recent myoc. infarction	(+)	++	P. r.
183. MU HR 1922/47	53 F	Diabetes mellitus. Hypertensive heart disease. Myocard. infarct. Gangrena pedis sin. (arterial thrombosis?)	+	+	P. v.
184. MU HR 2125/47	69 M	Coronary art. sclerosis Hypertensive heart disease Old myoc. infarction	+	++	A. v.
187. MU HR 2403/47	62 M	Hypertensive heart disease Coronary art. sclerosis Recent myoc. infarction	+	++ SD	A. v. P. r.
190. SSMI HR 2832/47	82 M	Hypertensive heart disease Coronary art. sclerosis Recent myoc. infarction	+	+++ D	P. r.

A. P.	Cong. heart fail.	Sito of atrial infaret.	Inf. age.	Heart rhythm.	Electrocardiogram
—	+	I. O. O. ?.	V	S/F	Two ECG's from the last 2 months. Aur. fibrillation.
—	+	O. II. III. ?.	R	F	One ECG 16 days a. m. auri- cular fibrillation.
—	+	I. II. O. O.	VR	S	Two ECG's from the last 2 months. See p. 84.
—	?	I. O. III. IV.	R	S	—
+	+	I. II. O. ?.	R	F	—
—	+	I. O. ? O.	R	F	One ECG 49 days a. m.: auric. fibrillation.
+	—	O. O. O. IV.	R	S	Three ECG's from 2 days a. m. Elevated P-Ta's in leads II and III (p. 94).
+	—	O. O. O. IV.	R	S	Three ECG's from 3 days a. m. Slightly elevated P-Ta's in leads II and III (p. 95).
+	—	I. II. III. IV.	R	S	—
+	—	(I). O. O. IV.	R	S/F	Two EGC's. 2 days a. m. elevated P-Ta's in leads II— III. ECG the day of death: auric. fibrillation (p. 94).
+	—	I. II. III. IV.	V	S	Short P-Q intervals after in- faret (see p. 91).
+	+	O. O. O. IV.	R	(F)	Two ECG's from tho 2 months before death, in both auric. fibrillation.
+	+	I. II. O. O.	VR	S	Two ECG's from the 2 last months before death. Sinus rhythm. Nothing abnormal with the atrial part of the ECG.
+	+	O. O. III. IV.	R	F	—



Case No.	Age Sex	Diagnosis	Hpt	Coron. scler.	Ventr. infarct.
191. SG HR 1425/47	62 M	Chronic valvular endocarditis. Aortic stenosis. Coronary art. sclerosis	+	++	A. v.?
193. SG HR 979/47	63 F	Coronary art. sclerosis Old myocardial infarcts Pulmonary art. embolias	—	++	A. v.
196. SSMI HR 2747/47	65 F	Coronary art. sclerosis Acute myocard. infarction	—?	++ D	P. r.
198. MU HR 759/48	69 F	Coronary art. sclerosis Acute myocardial infarction	?	++ D	P. r.
203. MU HR 351/48	65 M	Hypertensive heart disease Cancer ventriculi	+	++	—

(Case 203 was the only case without atrial thrombosis.)

A. P.	Cong. heart fail.	Site of atrial infarct.	Inf. age.	Heart rhythm.	Electrocardiogram
—	+	I. II. O. O.	R	S	Ono ECG 2 months a. m. No- thing abnormal with the atrial part of the ECG.
?	+?	I. II. O. O.	VR	S/F	One ECG 2 months a. m. sinus rhythm. Nothing abnormal with the atrial part of ECG. In an ECG 1 month a. m. aur. fibr.
+	—	O. O. III. IV.	R	S	One ECG 2 days a.m. Elevated P-Ta's in leads II and III.
+	+	O. II. III. IV.	R	S	One ECG 7 days a. m. Regular ventr. rhythm, P-waves barely visible.
—	+	I. ? . ? . ? .	VR	S	—

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# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CCXVIII (218)

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## THE STERNAL MARROW FUNCTION, WITH SPECIAL REFERENCE TO ERYTHRO- POIESIS, IN PERNICIOUS ANAEMIA

(WITH 8 FIGURES IN THE TEXT AND 28 TABLES)

BY

*HALL SCHARTUM-HANSEN*

Accompanies Vol. CXXXII (132)





FROM THE MED. DEPT. A. OF THE UNIVERSITY CLINIC, OSLO,  
NORWAY. CHIEF: PROFESSOR OLAV HANSSEN M.D.

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(SUBMITTED FOR PUBLICATION 1TH OF SEPT. 1947)

OSLO 1948



## FOREWORD.

This investigation was started in 1936, and during the four following years the material was collected and prepared at the Medical Department A. of the Rikshospital. Subsequently this material was added to from several other hospital departments. The conclusion and final revision of this work was rendered difficult by the war.

I wish to thank Professor OLAV HANSEN M. D., Professor HARALD SALVESEN M. D., the Senior Hospital Physician, ROLF HATLEHOL M. D. and the Senior Hospital Physician O. RÖMCKE M. D. for permission to use the material in their hospital departments. Most of this work was done at the Medical Department A. of the Rikshospital.

Altogether I have had occasion to undertake 271 sternal punctures on a total of 62 patients. I have also undertaken sternal punctures of 20 persons with normal bone marrow functions. They have served as controls.

I wish also to express my gratitude to the managers of the Medical Fund of the Freia Chocolate Factory Company, the managers of Dr A. MALTJE's Legacy and the managers of Professor O. Bang's Memorial Foundation for Economic Aid, as well as the managers of Nyegaard & Company for financial contributions to the costs of printing.

I owe Mrs STOLTENBERG a great debt of gratitude for the figures and diagrams she has drawn.



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## I. Introduction.

### *Pernicious Anaemia.*

It would seem that the first published account and complete description of a morbid condition presumably identical with pernicious anaemia were given by the Scot Combe in *Transactions of the Medico-Chirurgical Society* in Edinburgh in 1822. In the years which followed immediately, repeated references are to be found in English publications on a pernicious anaemia-like condition interpreted and described as a clinical entity. In 1851 BARCLAY mentioned glossitis in a patient dying of severe anaemia. In 1849 and 1855 ADDISON described a disease which he called »idiopathic anaemia» and which he associated with changes in the central nervous system as part of a syndrome.

Some years later, in 1867 and 1872, BIERMER described an anaemia which ran a fatal course and which he called progressive pernicious anaemia. He included in this term — contrary to ADDISON — a whole series of anaemias without clear-cut limits. A similar conception came gradually to be shared by several investigators including IMMERMANN (1874), QUINCKE (1876), and EICHHORST (1878).

From 1886 to 1909, WILLIAM HUNTER worked on this disease, maintaining like ADDISON that »Addison's anaemia» is a well-defined clinical entity involving the gastro-intestinal and nervous systems as well as the blood system. He drew attention to and emphasized the importance of the glossitis now known as Hunter's glossitis. He was the first effectively to distinguish this disease from other forms of severe anaemia.

I can find no justification whatever for calling this disease Biermer-Ehrlich anaemia — a practice common in the literature. From the outset so many investigators have made such fundamen-



tal and praiseworthy contributions to the study of pernicious anaemia that there is little justification for associating it with any one in particular, and absolute priority can be assigned to none. PEPPER in 1875 and COHNHEIM in 1876 were the first to describe megaloblastic bone marrow as an abnormality. In 1883 S. B. LAACHE was one of the first to maintain that the large, oval and haemoglobin-rich erythrocytes, whose size might be up to  $15\mu$ , are very characteristic of pernicious anaemia. His claims were based on examinations of native preparations.

EHRlich's introduction in 1881 of the staining of elements of the blood with aniline dyes was so masterly that one is tempted to regard it as the overture to a new era in haematological research. He interpreted the megaloblastic bone marrow of pernicious anaemia as indicating a degenerative process pathognomonic of this disease. Since then there have been two main and conflicting conceptions of the aetiology of this disease. HUNTER held that it is a sequel to intestinal sepsis and haemolysis of the portal blood, i.e. *abnormal blood destruction*. EHRlich maintained that the disease is due to the development of *abnormal blood corpuscles*. In spite of constant changes in our conceptions of the aetiology of pernicious anaemia as our knowledge of it has progressed, and even after the introduction of liver treatment, the controversy continues over the conflicting theories of abnormal blood formation and abnormal blood destruction. Our knowledge of folic acid and its place in pathology is also far from complete.

#### *Intravital Examination of the Bone Marrow.*

In 1868, NEUMANN and BIZZAZERO showed that the bone marrow is the seat of formation of blood corpuscles. In 1876 COHNHEIM gave a description of red bone marrow in pernicious anaemia. When the haematopoietic function of the bone marrow was discovered and some of its significance was thereby revealed, interest was aroused in its examination *intra vitam*, notably in the so-called diseases of the blood.

PIANESE, the first to take this step, punctured in 1903 the epiphysis of the femur in a search for malaria parasites. In 1908 GHEDINI trephined the tibia in order to examine the bone marrow,

and in 1921 ZADEK followed suit. In 1922 CARONIA, MORRIS and FALCONER, and in 1925 KRAMAR and HENSCH undertook punctures of the tibia, and in 1923 C. SEYFARTH trephined ribs and sternum. The same procedure was employed by WEINER and KAZNELSON, SCHILLING, BARTHA, INTROZZI, CUSTER, DAMESHEK, YAMAMOTO, PEABODY and others. However, none of these methods enjoyed any general approval, for they were too radical, veritable surgical operations totally unfitted for serial examinations of one and the same person.

In 1929, I. M. ARINKIN introduced sternal puncture with aspiration of sternal marrow. As this procedure is simple and not far-reaching, it has been introduced everywhere as a clinical method of examination. It has taken little time greatly to widen our knowledge of the haematopoietic function of the bone marrow, and it is the method adopted for the serial investigations in the present study.

In 1934, HENNING and KORTH expressed the opinion that ARINKIN's method is of little use as the material it yields for examination is scanty. They introduced irrigation of the sternal marrow with physiological saline solution as a better method in their opinion. Very probably they do not think so today. For their «diagnostic irrigation» of the sternal cavity has not attracted any attention.

## II. Plan of Work.

This work was started with the intention of following, by means of serial examinations of the yield of sternal puncture, the function of the bone marrow in the sternum in cases of pernicious anaemia before, during and after liver treatment.

Gradually the material became so large and so instructive in a single direction also with regard to our knowledge of erythropoiesis that I found myself justified in treating it as the basis for a discussion of the development of the erythrocytes and of the inter-relationship between the various forms of erythroblasts.

### III. Methods.

Whenever the number of examinations was small, the punctures were made in the manubrium sterni. When many punctures were made, they followed the corpus sterni downwards, and when necessary I continued with new punctures in the manubrium. No puncture was made in an old puncture opening even when up to 16 punctures were made in the same patient. The punctures were always at the level of the intercostal spaces in order to avoid any transverse strands of cartilage which are liable to be encountered at the level of the insertions of the ribs.

The punctures were made with a short, stout needle with a slightly slanting point, an internal diameter of 2 mm., and provided with a stylette. The cutis, subcutis and periosteum were always anaesthetized before a puncture by the injection of 2 to 3 cc. of parocain with adrenalin 1  $\frac{1}{100}$ .

After perforation of the 0.5 to 1 mm.-thick lamina externa sterni, the needle enters the 5 to 15 mm.-deep marrow cavity (ARIEFF). To create an adequate vacuum, I aspirate carefully with a 20 cc. record syringe. The aspirated blood, in a quantity I try to reduce to a minimum, is thereupon transferred to a micro-test-tube of quartz glass graduated exactly to 1/10 cc. and containing 0.1 cc. of a 3 % solution of calcium oxalate used to prevent coagulation. But first a couple of drops of sternal blood from the syringe are taken for smear preparations and reticulocyte preparations.

The smear preparations are stained in the usual way with May-Grünwald-Giemsa solutions and a differential count is made as with an ordinary blood smear from the ear. As a rule, I have counted 600 nucleated blood cells. CRUMBHAER and CUSTER as well as McLEOD consider it sufficient to count 500 nucleated blood cells. In the present work a greater number than 600 cells was

occasionally counted, particularly during normoblast crises when great quantities of normoblasts may be found. Hence the necessity for counting a greater number of cells than is customary in order to obtain a sufficient number of cells from which to calculate the numbers of erythroblasts relative to the other cells. The differential count never included less than 400 cells of the leucocyte type or less than 200 of the erythropoietic type.

The differential counts were made in such a way that I first began a differential count of all the types of nucleated blood cells in preparations. When I had reached either 200 erythropoietic cells or 400 leucopoietic cells, I discontinued the differential count for the time being. The percentage relationship between the two cell systems, recorded in the tables as percentages of erythroblasts and of leucoblasts, was thereupon calculated. I now calculated the percentage relationship to each other of the various cell forms in that cell system which had determined the preliminary cessation of the differential count. In other words, action was taken according to whether the differential count yielded 200 erythropoietic cells or 400 leucopoietic cells. At this stage the differential count was continued of the cells belonging to the other cell system, the count being continued either till at least 200 erythropoietic cells or at least 400 leucopoietic cells were counted. Thereupon the percentage relationship between the various cell types of this system was calculated.

To show the relationship between marrow particles and fat particles from sternal puncture as well as their quantity quite roughly, the following system of recording was adopted:

- 1) Normal content of marrow particles: ++ to +++
- 2) Normal content of fat particles: +++ to +++++

The patient's sensations in the sternal region during aspiration of the sternal cavity were also noted with each description of the puncture in the case records.

The reticulocyte preparations were stained with an alcoholic solution of Nile-blue sulphate and with the customary technique. Cell forms with remains of nuclei were not counted as reticulocytes, and I have also been wary over the inclusion of cells with single granules. The reticulocytes were not classed in groups, as for

example ad modum HEILMEYER. The leucocytes obtained from sternal puncture were counted in the usual way in a BURKER—TÜRK counting chamber and always twice. At these counts all the nucleated blood cells in the punctate came to be included in the count. In several cases the erythrocytes in the sternal blood were counted, and the haemoglobin was determined colorimetrically as acid haematin. The haemometers were corrected according to HALDANE so that 100 % corresponded to 13.8 g.% haemoglobin.

The serum colour was colorimetrically determined ad modum Meulengracht.

The various liver fractions referred to in this work were prepared by PER LALAND and AAGE KLEM in the course of their attempts to isolate the antipernicious anaemia factor. The terms given to these extracts are those employed by these investigators in their records. The efficacy of these preparations was estimated by their capacity to provoke the specific bone marrow changes of pernicious anaemia and by the effect of these changes on the number of blood cells in the peripheral blood.

Some of the patients were treated with the commercial liver extract of Nyegaard & Co. sold as Pernami.

## IV. The Genesis of the Erythrocytes.

This problem comes first. For the planning of the present investigation hinges on the morphology of the erythroblasts, their nomenclature and their relationship to each other. It is also this problem which has required a uniform and coherent handling of the findings and results of this investigation, and therewith the key to the comprehension of the following chapters.

### A. The Morphology and Nomenclature of the Erythroblasts.

Because of the divergent opinions on this problem and the consequent very diversified nomenclature, it is necessary first to give an account of the nomenclature to be employed in this work. This nomenclature has quite naturally developed in the course of the investigations, and will be gradually explained more fully. It is presented diagrammatically in fig. 1.

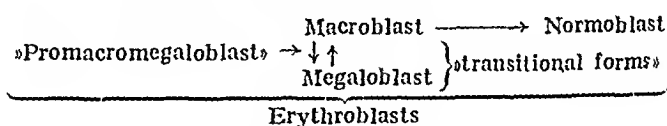


Fig. I. The Author's Nomenclature for the Nucleated Erythrocytes and Their Early Stages or Precursors

The «Promacromegaloblast» (Fig. VI A. b. pag. 85) (the pro-erythroblast of FERRATA, NAEGELI, the erythrogenie of HELLY) is of about the same size as the myeloblast, but is sometimes a bit larger, particularly in pernicious anaemia. The cytoplasm is dark blue, and its rim comparatively narrow. Cell and nucleus are circular, the latter comparatively small and often lighter than the cytoplasm. The structure of the chromatin is finely granular to finely

meshed. It is somewhat more dense than in the case of the myeloblast, with which it is most easily confused, and it is somewhat more coarse than in the case of megaloblast. As a rule, there are several nucleoli, generally circular, to be found.

All agree that this is a young erythropoietic cell, and most observers believe that it ripens into an erythrocyte through the macroblast stages. But from the observations made in the present investigation I believe that, in addition to being a precursor to the macroblast, this cell must also be regarded as a precursor of the megaloblast. For this reason I have found it natural to call it a *promacromegaloblast*. These cells are abnormally numerous in the sternal marrow in pernicious anaemia and congenital haemolytic jaundice (SCHARTUM-HANSEN). This numerical rise is to be interpreted as a response to a call for increased regeneration. In congenital haemolytic jaundice, promacromegaloblasts develop further through the normal erythroblast forms to spherocytes, whereas in pernicious anaemia they often degenerate through the megaloblast forms.

Probably this type of cell corresponds to the erythrogonic of ASKANAZY-HELLY and ELLERMANN, and the lymphoidocyte of PAPENHEIM.

According to NAEGELI and HENNING, there is a promegaloblast characterized by a still finer chromatin structure than is normally to be found in erythroblasts. The nucleus of this promegaloblast contains nucleoli, and the size of the cell is about 15  $\mu$ . It is easily distinguished from the «pronormoblast». Several other investigators (SCHULTEN and SEGERDAHL for example) find these distinctions uncertain. To judge by the description of this cell, it is, in my opinion, assuredly only a transitional stage between the promacromegaloblast and the more typical megaloblast.

According to TEMPKA and BRAUN, the difference between the «promegaloblast» and the myeloblast is that the chromatin of the promegaloblast nucleus is somewhat coarser and more dense, the nucleoli being less sharply defined. The shape of the nucleus is more even, and the protoplasm more basophile. They conclude that the «promegaloblast» is the larger.

ELSA SEGERDAHL also has seen «numerous and unmistakable» transitional forms between myeloblasts and «promegaloblasts»



which, in her opinion, show the same morphological characteristics as the early stages of the youngest normal erythroblasts, and which differ from them only by their size. The cell type described by these investigators is most probably identical with that described by NAEGLI and HENNING and already referred to.

Several other authors (FERRATA, HENNING, KEILHACK, ROHR) hold similar views and refer to a «promegaloblast» whose most distinctive feature is said to be its size, — larger than the «proerythroblast». They have, however, quite the same nuclear structure and cytoplasm. Further, they hold that the «promegaloblast» differs from the «proerythroblast» also in this that the former is oval and has a larger cytoplasmic rim in relation to its nucleus, is said to present a «cloudy pattern» and to have a more finely woven chromatin net. The cell thus described is, in their opinion, genetically different from the «proerythroblast». According to their description, this «promegaloblast» is wholly identical with the young megaloblast in this work and represents therefore a further stage in the development of the promacromegaloblast in a megaloblastic direction.

Lastly it should be pointed out that the promacromegaloblast of this work is quite certainly identical with what ROHR and NORDEENSON call a pronormoblast, and what SCHULTEN calls a basophile erythroblast and what recurs constantly in the American press under the misleading title of megaloblast.

The *megaloblast* (Fig. VI B. d. pag. 86) has been and still is a much debated cell because opinions differ about the cell structure of this type of erythroblast.

EHRlich was the first to give this name to a form of erythroblast, and it would be therefore natural to continue to reserve this name for types of erythroblast corresponding morphologically to EHRlich's first description.

This cell is plainly oval, polychromatic or hyperchromatic. According to EHRlich, the nucleus of these young megaloblasts forms is comparatively large, of a beautiful, finely threaded structure, often irregular or even patchy. In older cells there is every variety of transition to small, dark, structureless and often eccentrically placed nuclei which he held can often be expelled. The cell acquires haemoglobin early. The EHRlich-NAEGLI original con-

ception of the megaloblast as representing an embryonic retrogression of erythropoiesis was in later years refused by NÆGELI himself.

It may now be said that the typical megaloblast NÆGELI always maintained was pathognomonic of pernicious anaemia is more easily demonstrable in the sternal marrow and found in younger stages of its development than was the case with NÆGELI on examination of the peripheral blood.

It is probable that many of the forms of erythroblast originally described by NÆGELI as megaloblasts were often the cells described in this work as only *slightly* atypical macroblasts. The departure from the typical is slight because the cell is exposed to only a slight shortage of the supply of the antipernicious anemia factor to the cell. This last-named type of erythroblast is very easily found in pernicious anaemia particularly when it is somewhat advanced. It is therefore apt to be interpreted as a specific type of erythroblast, but it is not identical with the really 'quite typical megaloblast as originally described by EHRICH, and it is somewhat coarser in nuclear structure. Morphologically it resembles some of the types of young erythroblast found in the sternal marrow in the marked macroblast anaemias of a non-pernicious type. But we must not forget that NÆGELI's conception of the megaloblast's morphology was in the main founded on examinations of the peripheral blood. His views in this matter were shared by most earlier haematologists.

In my opinion, the young megaloblast is usually larger, to some extent much larger, than the young promacromegaloblast to which it is genetically related. Its size is from 13—14 up to 20 and, occasionally, up to even 30—40  $\mu$ . These giant cells are called gigantomegaloblasts. Some, particularly those of the older type, are oval. In the youngest types of megaloblast, the plasma brim is narrow, but it becomes gradually wider. The colour ranges from dark blue in the younger forms to light blue in the older. Later in their development, which I interpret as degenerative on account of waning formation of the antipernicious anemia factor, there is a further change in some of the megaloblasts. Their cytoplasm becomes more or less eosinophile, and their nuclei undergo pyknosis to some extent. But most megaloblasts probably die before reaching

this stage. These forms of erythroblast, particularly the most fully developed types with a morphology as described above, are identical with the megaloblasts originally described by EHRLICH and NAEGELI who held that they were demonstrable only in pernicious anaemia. This opinion has been confirmed by most later investigators, and I have also failed to find erythroblasts with the above-mentioned morphology in patients not suffering from pernicious anaemia, although I have examined by sternal puncture hundreds of patients suffering from macroblastic forms of anaemia.

A further characteristic of a typical megaloblast concerns the structure of its nucleus which is evenly homogeneous, finely granular with now and then remains of the nucleoli of a promacromegaloblast. The position of the nucleus is often eccentric, particularly in the older types. In smears from sternal punctures of my cases I have repeatedly noticed that the megalotypes seem to be more vulnerable as the cells grow older than the other types of erythroblast. This seems to be a sign of degeneration.

KLIMA maintains, and ROHR believes he has seen that the older, more mature megaloblast nucleus has an irregular »geflecktes, schickiges» appearance. It seems to me that the cell they describe must therefore be regarded as a macroblast, somewhat pathological on account of only slight failure of the antipernicious anemia factor. From the account given of its morphology, it is essentially different from the pure megaloblast resulting from complete failure of the supply of the antipernicious factor to the cells.

In 1935, TH. GÜSE described a case of pernicious anaemia in which there were large, unripe basophile cells in the peripheral blood in addition to megaloblasts. These basophiles were demonstrable post mortem in the bone marrow and several other organs. GÜSE regarded this form of cell as a basophile precursor of the »megaloblast series». To judge by the description of its morphology, I take it to be an ordinarily large, possibly giant megaloblast.

O. JONES claims to have found azurophile granulations in the »promegaloblasts», — a type of cell he believes to be an intermediate stage between basophile megaloblasts and the myeloblast. ELSA SEGERDAHL has also found granules in the megaloblast, but she thinks they may possibly be artefacts.

*The Macroblast* (Fig. VI C. c. pag. 87) (NAEGELI) — *normo-*

*blast* (Fig. VI D. e. pag. 88) (EHRlich). Under normal conditions, these cells, with all their transitional forms, constitute the whole developmental series between the promacromegaloblast and the ripe erythrocyte.

The youngest forms of macroblast are as large as the promacromegaloblast. They become gradually smaller as they ripen till at last their size is that of an ordinary eosinophile normoblast, i.e. a little larger than an ordinary normocyte. The cytoplasm of the macroblasts ranges from dark to lighter basophile. In certain phases of pernicious anaemia, eosinophile macroblasts are to be found. In health they retain their basophile properties till they reach the normoblast stage. The nuclear structure of the macroblasts is more coarsely granular than that of the promacromegaloblasts and the megaloblasts. This is more and more the case as the cell grows older; as it does so, its nuclear structure, beginning with that of a promacromegaloblast, becomes constantly more firm and coarse, until, through all the transitional stages, it finally goes over to the normoblast's spake-shaped arrangement of the coarse clumps of chromatin. A normoblast is a little larger than an ordinary normocyte. Cell and nucleus are circular, and the cytoplasm ranges from basophile to oxyphile. The nucleus undergoes pyknosis gradually. Thereupon the normoblast discards its nucleus either by karyolysis or karyorrhexis. Through the reticulo stage it then becomes a ripe erythrocyte. There is assuredly no reason for confusing a normoblast with other erythroblast types.

*Conclusion:* The morphology of the erythroblasts and the nomenclature of the various types of erythroblast are described and discussed. The following are a few points which are of special interest and which are of significance to the further development of this work:

1) A «promacromegaloblast» is, as its name implies, a precursor of all the erythroblast types.

2) The megaloblast, as defined by EHRlich and NAEGELI, is pathognomonic of pernicious anaemia.

3) As compared with other erythroblast types, the megaloblast as it grows older shows an increasing tendency to be easily damaged in smears. Is this a sign of degeneration?

## B. The Genetic Connexion of the Various Forms of Erythroblasts.

1) *Previous Investigations.* It is assumed that erythropoiesis is medullary from the sixth month of foetal life, and that the development of the normal erythrocyte proceeds from the original or parent cell through a »proerythroblast stage». Thereafter the proerythroblast develops through a macro- and normoblast stage before the nucleus disappears and the normal erythrocyte comes into being. Most haematologists agree in the main on this score and concerning the morphology of the older types of this series of erythroblasts. But the nomenclature may vary considerably and therefore interfere with the comparison and appreciation of the results of different investigators. This was pointed out in the preceding chapter also (see VEENEKLAAS for example).

We do not yet know what is the source of the youngest erythroblast forms. In 1912, NAEGELI suggested that they might be derived from:

- 1) Undifferentiated »mesenchyme cells».
- 2) Endothelial cells or the endothelium of blood vessels.
- 3) Lymphocytes.

It is in the main the same possibilities which are discussed today. We still lack a relatively unimpeachable explanation of these problems, and in the present work I have also failed to find a basis for a really well-founded and plausible opinion on the subject. There are many different theories, but most haematologists today assume that the erythroblasts, like the myeloblasts and megakaryocytes, are derived from the reticulum cells of the bone marrow or the »bone marrow mesenchyme».

DOAN and WISEMAN have recently maintained that the erythrocytes are derived from the endothelium of the blood vessels in the bone marrow, and that they are formed within them from the so-called »intrasinusoidal capillaries». They hold that, under normal conditions, the »megakaryoblasts» are formed from this endothelium. Thereafter they develop into »erythroblasts» and further into normoblasts. But these investigators believe that the formation of the leucocytes in the bone marrow is extravascular. Other investigators such as SABIN and CUNNINGHAM (chicken experiments in 1920) are

of the same opinion. From another starting point, about the turn of the century, SCHRIDDE and NAEGELI held that the megaloblasts are the first stage in the development of the erythroblasts, being derived from the endothelium of the blood vessels. These investigators did not find megaloblasts under normal conditions.

With O. P. JONES, I must, however, say that SABIN and CUNNINGHAM and many American investigators have defined the megaloblast in terms quite different from the original conception of it. This is calculated to create confusion. Besides, it is difficult to understand the reason for, and object of, this new definition. I assume that the megaloblasts of these investigators are not the outcome of a misinterpretation of the morphology of these cells which, to judge by their description of them, correspond to the promacromegaloblasts in my study.

This confusion does not diminish when these investigators let their megaloblast develop into a «normoblast», described by them as smaller than the megaloblast, with a somewhat denser nucleus, with somewhat less basophile cytoplasm, and with a capacity for division. The «normoblasts» thereupon proceed to develop into «erythroblasts» with a pyknotic nucleus, and with the disappearance of basophilia at this stage. Their «normoblast» is, to judge by their description of it, the same cell as that named macroblast in my work, possibly also the same as younger normoblast types. «Erythroblast» corresponds to the fully developed normoblast in my work.

Several investigators claim to have found «megaloblasts» in greater or smaller quantities in normal persons (YOUNG and OSGOOD in 1935). HOLMES and BROWN, for example, found in 1930 in seven normal persons an average of 5.2 % «megaloblasts» among the nucleated blood cells obtained by sternal puncture. Of the erythroblasts, representing 12.1 % of these nucleated blood cells, the remaining 6.9 % were «normoblasts».

But most investigators have not found megaloblasts or, more correctly, the erythroblast types which they call megaloblasts, in normal sternal marrow (HENNING, KEILHACK, FORSELL and many others).

In 1937, in a not very convincing publication, SCHULTEN maintained that the megalo types and macro types are not derived from

two fundamentally different cells, but from one and the same cell, i.e. from the cell discussed by myself in the same year as a pro-macromegaloblast. But SCHULTEN, at variance with myself, maintained that the megaloblast and macro types follow two different courses in their development.

THADDEA and BAKALOS maintain that the two erythroblast types (the megaloblast and macro types) are cells which run a completely parallel course and undergo a completely different erythropoietic development, — the «normoblastic» and the «megaloblastic» series. Thus in this matter they agree with NÄGELI, ROHR, DEAN, PINEY, SCHILLING, MURPHY, NORDENSON and many others.

Other investigators may say the same thing quite simply in another way, — that the megaloblast series develops from the youngest erythroblast forms, in response to an inadequate supply of the antipernicious factor (DAMESHEK, VALENTINE, PEABODY, ISAACS, HENNING).

After investigating goats' milk anaemia in children, VEENEKLAAS has suggested that there are two possibilities of development for the large, young erythroblast types, one through normoblasts to normocytes, one to «megalocytes».

Other investigators follow quite different lines in their nomenclature. ISRAELS, for example, formulates the following erythroblast types: The haemocytoblast, the proerythroblast, megaloblasts types A, B, and C, as well as normoblasts types A, B, and C. He formulates a scale of development of the erythroblast forms based on the following nomenclature (fig. 2).

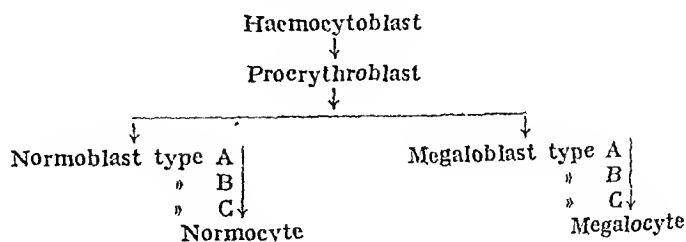


Fig. II. Diagram of the development of the erythrocytes according to Israel.

He gives the following description of the cell forms mentioned: The haemocytoblast, original or parent cell, is large; and has a comparatively large nucleus occupying most of the cell. The brim of the cytoplasm is small and blue. The nucleus is a somewhat

lighter blue and has a blurred nuclear structure. There are two or more nucleoli in the nucleus. The proerythroblast is the youngest cell which certainly belongs to the erythropoietic system. The cell is large and its large nucleus has a clearly marked structure. There are several nucleoli in the nucleus. The megaloblast type A is smaller than the proerythroblast and has dark blue cytoplasm. There are no nucleoli in the nucleus which has a characteristically open chromatin net. The nucleus of the megaloblast type B is shrunken and has a network which is finer and still more open. The cytoplasm contains haemoglobin. The nucleus of the megaloblast type C occupies an eccentric position, is small and pyknotic. This nucleus lacks a pattern or has only a suggestion of one.

The normoblast type A is large but somewhat smaller than the megaloblast type A. Dark blue cytoplasm forms a ring round a large, circular nucleus. There are no nucleoli in the nucleus whose chromatin is distributed in haphazard fashion in dark clumps separated from each other by lighter areas. The normoblast type B is small, only a little larger than a normal erythrocyte. The nucleus occupies only about half of the cell. The chromatin is collected in clumps sometimes distributed like the spokes of a wheel. The colour of the cytoplasm shifts from blue through grey-blue to red. The normoblast type C is small, of the size of an ordinary erythrocyte. The pyknotic nucleus shows no definite structure. According to ISRAELS, the megaloblasts, particularly those of types B and C, are associated quite as a matter of tradition with pernicious anemia. The normoblasts are precursors of the normal erythrocytes.

ISRAELS' nomenclature and classification seem unnecessarily artificial and «schematic», and are so foreign to the usual classification of the erythroblasts that there is little point in building further on these.

2) *Own Investigations.* When we study the erythropoietic cells, notably those associated with the so-called hyperplastic conditions, we find a veritable swarm of cell types. Since the introduction of liver treatment by MINOT and MURPHY in 1926 subsequent to the preliminary work of WHIPPLE, and above all since the introduction of sternal puncture as a clinical test, we have been so enriched by the material available for investigation that we have gained a much improved insight into the function of the bone marrow.

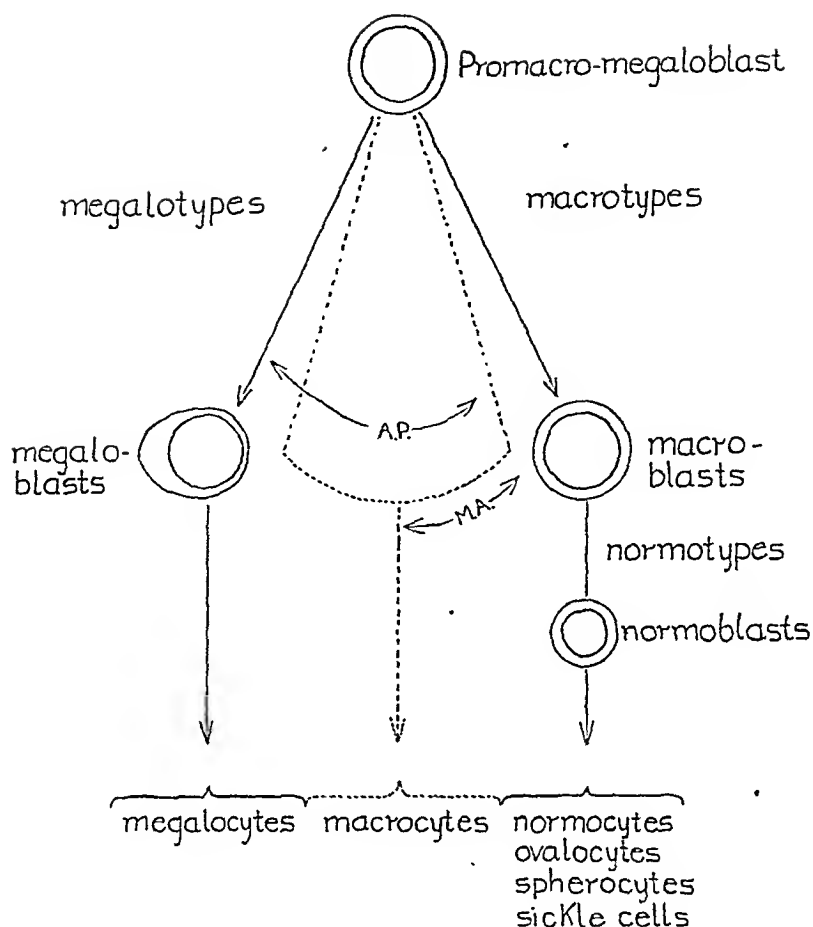


In the present work, serial investigations of smears from the sternal marrow in cases of pernicious anaemia have led me to feel justified in claiming that I have discovered the process of development which the erythroblasts undergo normally and in this disease. This development points to the sequence: normocytes, macrocytes, and possibly megalocytes. All the various types of erythropoietic cells are intimately connected with each other and form an indestructible entity from the developmental point of view, both in health and in all the types of anaemia and diseases of the blood. This entity is characterized by innumerable *transitional forms* (Fig. V A. a. pag. 83) between the types of erythroblast discussed in the previous chapter IV A. The notion that there are two different developmental series should be completely discarded, and it is most probable that all the erythrocyte types are developed from the same parent types and through the same forms of erythroblast which, may, however, vary somewhat morphologically if there is a shortage or total lack of certain substances necessary for their ripening into normal erythrocytes.

The above-mentioned *transitional forms* render difficult the classification of erythroblast forms in well-defined, distinct types. In other words, we cannot in most cases say whether a given cell is a typical macroblast or a typical megaloblast or, in a wider sense, is a well-defined form of erythroblast. What we can say with certainty is that such and such a cell for example resembles morphologically a megalocyte type in an early stage or a macro type in some other stage, or one of the other forms of erythroblast. Such a standpoint is also the most natural. Here, where we have only one coherent process, it would be impossible to say whether such and such a cell belongs to a fixed place in our scheme. This would also not be very satisfactory from a physiological point of view.

In the differential counts of bone marrow smears in the present work, the percentages refer to the relative proportions of the cells which most closely resemble the main types already described and figuring in the framework of our tables (see fig. III).

The youngest cells which in the present work could with certainty be characterized as erythropoietic are the promacroblasts already described. When the supply of the antipernicious anaemia factor is normal, and when the conditions for erythrogenesis are



*Fig. III.* The author's diagrammatic presentation of erythropoiesis with the development of all the erythrocyte types. It also shows the interrelationship of all the known erythroblast forms. In the sections bounded by a broken line are the macromegaloblastic transitional forms.

A. P.  $\longleftrightarrow$ : The reversible process in pernicious anaemia.  
 M. A.  $\longleftrightarrow$ : The reversible process in macrocyte anaemias.

in other respects in a state of equilibrium, this cell develops through the normal macroblast stage to the normoblast, i.e. through the macro and normo types to the right in fig. III.

When the supply of the antipernicious anaemia factor fails, one may assume that there is a morphological change in development, and that the erythroblasts degenerate more and more and approach the megalotypes in appearance, i.e. come nearer and nearer to the cell forms to the left in fig. III, following the arrow A.P. Now we must, however, in pernicious anaemia, as in diabetes

pellitus for example expect differences in the supply of hormone, to organs and individual cells. Here I class insulin and the anti-pernicious anaemia factor together as hormones to indicate that in pernicious anaemia one may expect to find various forms of erythroblast whose occurrence depends on the degree and development of this disease. In theory one should then expect the marrow in cases of «total pernicious anaemia» to be composed exclusively of promacromegaloblasts and the megaloblast types in their most advanced forms as they must be supposed to belong to that type of cell which, in its pure form, appears when the supply of the anti-pernicious anaemia factor to the cell fails completely. A description of such a fully developed form of pernicious anaemia is not to be found in the literature nor in my own records. Indeed, in my opinion a pure megaloblastosis is incompatible with life. Hardly any of the large erythrocytes in pernicious anaemia can have been derived from megaloblasts for, as already pointed out, these cells probably degenerate and do not turn into erythrocytes (see also my case records in which no eosinophile megaloblasts practically were to be found in differential counts). Consequently, the production of erythrocytes would cease completely when there was «total megaloblastosis». As this point of view is completely at variance with what is commonly accepted, it must be explained more fully.

The large megalocytes have been held for half a century to be derived from the megaloblasts (NÆGELI, LEITNER). My own sternal punctures have, however, led me to believe that the large erythrocytes in pernicious anaemia are derived from the macroblasts, particularly from the transitional forms of macromegaloblast, and must therefore be regarded as macrocytes. The larger an erythrocyte is, the closer is it related to the megaloblast types (see fig. III). The pure megaloblasts do not develop into megalocytes, but degenerate at an earlier stage as already pointed out; and a sternal marrow consisting exclusively of megaloblasts does not exist for obvious reasons. As long as the sternal marrow in pernicious anaemia contains erythropoietic cells, i.e. cells of the macroblast type, relatively capable of reproduction, a certain number of erythrocytes will be formed although they will, to be sure, be pathological. Among them the macrocytes will dominate, and this will be so more and more as the anaemia develops and the macro

types in the sternal marrow acquire a megaloblastic character more and more, approaching the megaloblast type morphologically. The marrow will gradually become less productive although the erythroblast count is comparatively high (see later chapters). This again shows that the more the erythroblasts resemble the megaloblast types, the smaller will be the quantity of these cells developing into erythrocytes.

If we consider the development of erythrocytes in pernicious anaemia from such a point of view, it will be easier to understand the morphologically different cell types in bone marrow and their relationships among themselves. Morphology and development will lose that taint of «schematism» to which a purely diagrammatic presentation so easily exposes them.

Sternal puncture in untreated cases of pernicious anaemia yields many different types of erythroblast. Some of them, as my own material shows, are fairly typical megaloblasts, whereas most of them are transitional forms of megalomacroblasts (see my case records in which these last-named cells are referred to as macroblasts). These transitional forms occupy a position morphologically between the normal macroblast and the typical megaloblast, and the type is determined by the degree of failure of the supply of the antipernicious anaemia factor to the cell.

As far as the evidence of my material goes, it would seem that, as already pointed out, the typical megaloblast undergoes degeneration. The megaloblasts can therefore hardly give rise to megalocytes which can best be described as belonging in theory to the erythrocyte types, and this is the more likely as it seems to be more than doubtful whether quite definite eosinophile megaloblasts are to be found (see case records). At any rate there is a great numerical preponderance of macroblasts — macrocytes over megaloblasts and the few macrocytes which are so large and oval that they might possibly be confused with megalocytes.

In my study of the cell types in the various stages of pernicious anaemia, I have come to the conclusion, as already pointed out, that I am justified in maintaining that in this disease the large erythrocytes *must* be derived from the macroblasts and not from typical megaloblasts. The size of the erythrocytes depends on the closeness of their nucleated precursors to the megaloblast types. The more they

develop to the left in fig. III, the greater will their size be. The few oval erythrocytes usually to be seen should be regarded as old macrocyte forms. In an earlier work on oval erythrocyte forms, I claimed to have shown that older erythrocytes very probably tend to assume an oval form.

The arguments in favour of the views expressed in this chapter will be supported as exhaustively as possible in the further study of my material.

CORTI and CIBOLDI, BRUGSCH and WINTROBE maintain that the increase in the average size of the erythrocytes during the crisis following liver treatment suggests that megaloblasts ripen into megalocytes. In the first place it seems very unlikely that the few megaloblasts found could give rise to the many «megalocytes» in smears from peripheral blood in pernicious anaemia. In my cases 54 and 59, for example, the basophile macroblasts were seen during a short period to develop into eosinophile macroblasts which most naturally and most probably should be regarded as precursors of eosinophile macrocytes. Later on in the regeneration period of pernicious anaemia, the basophile macroblasts develop as usual through normoblast stages into normocytes. In the second place it is natural that the average size of the erythrocytes rises when the small erythrocyte types, the schizocytes, the microcytes and most of the cell variations of anisocytosis disappear. It is also obvious that the great number of reticulocytes thrown into the peripheral blood during the crisis must raise the average size of the erythrocytes as long as the intense new formation and change in the composition of the bone marrow are proceeding. In health the erythrocyte is, to be sure, smaller than the reticulocyte. It is also very natural that, during this intense period of regeneration, a certain number of macrocytes should slip out from the bone marrow on a macroblast basis. Note in this connexion PERSON's statement that normal reticulocytes are from 1.12 to 1.15 times as large as a normal erythrocyte, and, in pernicious anaemia, from 1.21 to 1.29 times as large as a normal erythrocyte.

*Conclusion: All the erythrocyte types are developed from the same erythropoietic cell types in the sternal marrow and through the same erythroblast forms. These erythroblast forms, for example the megalocyte types and the transitional, macromegaloblast forms in per-*

*pernicious anaemia may vary morphologically if they lack substances necessary for their ripening (fig. III). The large erythrocytes of pernicious anaemia are derived from macroblasts and transitional macro-megaloblast forms, and they must be defined as macrocytes from the morphological and genetic points of-view.*

## V. The Normal Sternal Marrow.

1) *Previous investigations.* In 1868, NEUMANN gave an account of the bone marrow as an organ, and in the same year BIZZAZZO described its wealth of blood vessels. But, to judge by the literature, no one seems to have investigated the interrelationship of the various types of cell in the bone marrow or the absolute number of nucleated blood cells before WOLOWNIK did so in 1905. His smears were obtained post mortem from the marrow of 60 patients who had died of sepsis, tuberculosis, pneumonia, cirrhosis of the liver, nephritis, heart disease or tumours. He found that the erythroblasts constituted 0.6 to 5.6 per cent. of the nucleated blood cells. In 1910, I. LOSSEN put this percentage at 10.7, after emulsifying and shaking up with glass beads marrow pressed out of the ribs of the cadaver. His counts of nucleated blood cells ranged from 270,000 and 1,568,000 per mm<sup>3</sup>. In 1915, SCHILLING and BENZLER examined the marrow of the cadaver and found the erythroblasts were, on the average, 36.6 per cent. of the nucleated blood cells, the range of variation being between 31.6 and 42 per cent. Twenty years later, DAMESHEK put this percentage at 50, and in 1937, ISAACS found 900,000 to 1,000,000 nucleated blood cells per mm<sup>3</sup> in the marrow of the cadaver. As we shall see later, these figures are much higher than those yielded by sternal puncture.

Naturally counts from cadaver marrow treated in different ways cannot be very exact nor give concordant findings. This is why so few such investigations are to be found in the literature.

When sternal puncture was introduced, its advantages became apparent, and it led to a growing demand for more knowledge of the normal marrow. During the last 15 years, a whole series of investigations has increased our knowledge of the composition of the normal sternal marrow.

The sources of error in the quantitative and qualitative estimates of the bone marrow are still great, the greatest being the admixture of blood. For sternal puncture yields sternal blood as well as the extravascular cells of the sternal marrow. This sternal blood is drawn during aspiration from capillaries and mixed with the extravascular cells of the bone marrow in variable quantities. Sternal puncture also yields whole particles of connective tissue and fat as well as blood from the «intersinusoidal capillaries». The greater the quantity aspirated, the greater the admixture of blood, and the smaller the number of the cells proper to the bone marrow. All who have practised sternal puncture have quickly found this out and have therefore sought to aspirate as little sternal blood as possible. From 0.1 to 0.3 cc. is usually regarded as the most suitable quantity to aspirate.

It may be noted that when the sternum was trephined, the youngest cell forms were found to be relatively more numerous than they are now on sternal puncture (LEITNER).

To show how the number of nucleated blood cells in sternal marrow diminishes between the first and later drops of aspirated blood, TUCHINSKY and KOTARLENKO have examined patients suffering from typhus, and have found the first drop to contain from 23,700 to 223,200 nucleated blood cells per  $\text{mm}^3$ . After the aspiration of 5 cc. of blood, this number fell to 10,600 to 27,500. In his 12 cases, GREIF found a reduction between the first and fifth drops of from 8,000 to 94,000, with an average of 38,200 per  $\text{mm}^3$ .

On the other hand, conditions are very commensurate when the technique is uniform. COTTI seems to be quite right in saying that if only one keeps to the first drop of blood, conditions are practically constant.

There is also a tendency for many to believe that the sternal marrow reflects the conditions in the whole of the bony system (COTTI, REITER, NORDENSON, STODTMEISTER and BUCHMANN, SCILLING, YAMAMOTO). This possibility that the sternal marrow is representative of the marrow of the whole bony system as the creative source of the blood cells opens up wide perspectives in our interpretation of the findings of sternal puncture. In a healthy person, killed by accident, STASNEY and HIGGINS found in «ab-klatsch» preparations the same morphological conditions in the



sternum, ribs and vertebrae. Much fat may be found in hypoplastic conditions and may impair our judgment (REITER). Others also have maintained that, under pathological conditions, sternal marrow can be inhomogeneous (HELPAP, JEANNERET, DOMARUS).

T. S. HANSEN found morphological conditions in sternal marrow to be constant at various ages, from 11 to 70 years. On the other hand, SEGGER and REITER found that sternal marrow becomes poorer in cells and richer in fat with increasing age.

STODTMEISTER and BUCHMANN have shown that sternal punctures, even when they are repeated very often, have no effect on the composition of the cells in the blood. In serial investigations of the same persons, NORDENSON and MALLARME found conditions to be constant. GREIF has shown that, when sternal puncture was carried out at points near to each other in the horizontal line, the number of nucleated blood cells ranged between  $\pm 0.4$  and  $\pm 14.4$  per cent., and at different levels with less than  $\pm 30$  per cent., counts at different times ranging from  $\pm 1.2$  to  $\pm 11.8$  per cent.

Table 1 gives the findings of several investigators in respect of nucleated blood cells and the percentage of erythroblasts in relation to them on the sternal puncture of normal persons. I have been unable to compare my own material with some of the findings published by other investigators as the technique of their counts and differential counts has departed too much from that usually employed by others as well as myself. VIDEBAEK, for example, bases his calculations on the relationship of the various cell types to the »specific» marrow cells. He has hoped in this way to eliminate the influence of any admixture of blood on his figures. His system gives an artificial impression and is somewhat obscure. For in the first place one must assume that in normal sternal marrow there are always some types of leucocyte which belong to the peripheral blood and which do not hail from the capillary blood. In the second place we may have to form an opinion in a series of conditions in the bone marrow in which a relatively great number of the older nucleated types of leucocyte is to be found, as for example in chronic myeloid leukaemia and leucocytoses of different aetiology.

On the whole, the figures in table 1 are remarkably concordant when one remembers that the various investigators have used a

Table 1. A survey of the normal material of several authors with regard to the number of nucleated blood cells found on sternal puncture and the percentage of erythroblasts among them.

	Range of nucleated blood corpuseles		Erythroblasts per cent		Number of persons
	Mean	variation	Mean	Variation	
ARINKIN 1929 ....			11.—	6.5 —18.9	
BARTA 1931 .....				30.—33.—	
ESCADERO and VARELA 1932 ..			27.—		
SEGERDAHL 1935 ..	75,000	10,000—250,000	12.—	0.—31.—	52
NORDENSON .....			17.2		38
PICENA 1937 .....			18.3		
J. MALARME 1937 ..			16.—		
YOUNG and OSGOOD				5.4 —20.—	
GEFIF 1938 .....		45,000—150,000		10.—25.—	104
HOLMES and BROUN 1933 ....			12.1		7
WEIL and PERLES 1938.....			20.—		
CUSTER and KRUMBHAAR .....		Trephine marrow	32.2		
KLIMA 1938 .....			27.2		
ROHR .....			30.—	13.4 —43.—	10
HENNING and KEIL HACK 1939 ....			29.7	19.3 —40.4	10
SCHIARTUM-HANSEN 1940.....	71,700	25,500— 95,200	15.8	8.—24.2	7
LEITNER 1942 ....		60,000—100,000	25.3		22
T. HANSEN 1941 ..	113,700	34,400—301,200	15.—	4.2 —30.4	66
KILNLE 1943 ....			15.6		18
MARKOFF 1937 ....			25.4		20
SCHILLING .....			13.1		
WEINER and KAZ- NELSON 1926 ....		Trephine marrow		31.—44.—	
FORSELL 1939 ....			25.6		
PITTS and PACKHAM 1939 <sup>1</sup> .....	23,000	7,550— 46,000		16.7 —20.—	20
			9.5	2.3 —15.6	24 asp. 10 cc. blood
VOGEL and BASSIN 1937.....	118,000				
YOUNG and OSGOOD 1935.....			21.7	10.8 —33.3	28
TÖTTERMANN 1939..			16.2		

<sup>1</sup> Lymphocytes were not included in the calculation of the erythroblast percentage with the result that this figure is a little too high.

diversified technique in obtaining their material and in classifying and calculating the number of the different cells. There have been variations in the amount of blood used for counts, in the choice of first or later drops of blood, and in the counting of the nucleated blood cells.

It will be noted that very few investigators have counted the nucleated blood cells from sternal puncture. This is assuredly so because of the general belief that this count is of little value on account of its wide range of variation. A glance at table 1 shows that the numbers of the nucleated blood cells are well within the normal limits of variation as set by ELSA SEGERDAHL. It is natural that PITTS and PACKHAM have found such low figures as they aspirated 10 cc.

The table shows that the normal range of the erythroblasts is from 0 to fully 40 per cent. These extreme limits, to judge by my own experience and that of most other observers, are too far apart. More reasonable limits are 15 to 20 per cent.

We may surely conclude that the sternal marrow findings in normal material by most observers are so well in agreement with each other that they can serve admirably as a criterion by which to detect pathological conditions. KRUMBHAAR and CUSTER say outright that a careful differential count of bone marrow smears gives »true information regarding the status of the haemopoietic tissue». The conditions sometimes difficult to interpret are the hypoplasias in which the findings may be somewhat dissimilar because the bone marrow may be inhomogeneous. This is also my experience. But in the »hyperplasias» it would seem that one can expect to find stable conditions in the marrow of the sternum and other bones.

## 2) *Own investigations and an valuation of sternal puncture as a methou.*

To check up on the literature quoted and on my own observations so favourable to the value of sternal puncture, I have extended my investigations to 20 other persons representing normal material. They were patients in the Medical Department B of the Rikshospital and their peripheral blood and blood sedimentation test were normal. They suffered from such »benign» diseases as ischial-

Table 2. The author's sternal puncture normal material. Average figures and range of variation of the numbers of the nucleated blood cells and of the differential counts from smears (20 cases).

				Mean	Variation
Erythropoiesis	Erythroblasts		per cent	16.3	9.3 —22.5
	Leuco-	blasts	»		
		cytes	»	87.7	77.5 —90.7
	Pro-	megalobl.	»		
		macrobl.	»	0.80	0. — 3.
	Megalobl.	bas.	»	—	—
		eos.	»	—	—
	Macrobl.	bas.	»	6.65	3 —11
		eos.	»	0.35	0 — 3
	Normobl.	bas.	»	72.80	41 —89
		eos.	»	17.—	6. —36.
	Erythrobl.	bas.	»	0.40	0. — 3.
		eos.	»	0.15	0. — 2.
	Erythrobl.	bas.	»	1.50	0. — 3.
	division forms	eos.	»	0.35	0. — 3.
Leucopoiesis	Megacaryocytes		»	0.14	+ — 0.75
	Mast	immature	»	0.20	0 — 0.50
	cells	mature	»	0.09	0. — 0.75
	Eos.	myelocytes	»	1.61	0.25— 3.75
	Leucocytes	band forms	»	1.36	0.25— 2.75
		polymorphs	»	0.73	0. — 2.25
	Myeloblasts		»	2.21	0.50— 4.50
	Praemyelocytes		»	4.90	1.50— 9.75
	Neutrophiles	myelocytes	»	14.68	8.75—23.50
		young forms	»	29.60	22.25—37.50
		band forms	»	12.10	8.25—16.50
		polymorphs	»	9.98	4.75—17.25
	Mono-	blasts	»	} 1.58	0.50— 3.25
		cytes	»		
	Lymphocytes		»	16.08	9.25—20.25
	Plasma & Türk cells		»	0.91	0. — 2.25
	Smears cells		»	3.62	0.75— 7.25
	Reticuloendothelial cells		»	0.21	0. — 1.
	Nucleated blood corpuscles			98,290	(20,000) 39,300—218,400

gia, gastric ulcer without haemorrhage, and other ailments not likely to involve the blood-forming organs.

Table 2, which deals with my own normal material, shows that the figures for the nucleated blood cells yielded by sternal puncture

were within the limits indicated in the literature (see table 1), the average of 98,300 cells per  $\text{mm}^3$ . being close to the figures of other observers. The normal range of variation must be put at 20,000 to 30,000 and up to fully 200,000 nucleated blood cells per  $\text{mm}^3$ . These figures should be a very useful guide to pathological conditions. Table 2 also shows that my erythroblast findings tally well with those of other observers, my average being fully 16 per cent., and range of variation from 9 to 22.5 per cent., — useful guides to the study of pathological conditions.

Table 28 (pag. 112) deals with single examinations of normal material. From the description of the punctures it will be seen that they were all associated with a moderate degree of aspiration pain. A macroscopic examination showed numerous particles of marrow and fat in about equal quantities, with some preponderance of the latter. The aspiration in nearly all these cases proved easy. The maximum vacuum required was that obtained when the piston of the syringe was drawn back to the lines marking 10—15 cc. The first puncture of patient XI in my normal material showed quite extraordinary results which were not included in the calculation of the average range of variation. This was evidently one of those rare cases in which a focus of fat was aspirated. No discomfort was experienced during the aspiration which yielded only numerous particles of fat mixed with a little blood. The differential count showed a relatively great number of neutrophile leucocytes with segmented nuclei indicating a liberal admixture of capillary blood. The small number of nucleated blood cells (10,000) per mm. pointed in the same direction. A new sternal puncture yielded 91,100 nucleated blood cells per  $\text{mm}^3$ , the sternal marrow being normal also in other respects.

Table 2 also shows the average figures of differential counts of sternal puncture smears. Here, too, there is good agreement with the summary of the counts from each patient in table 28. With regard to erythropoiesis, it will be noted that, under normal conditions, there are here and there a promacromegaloblast, a small percentage of macroblasts, and numerous normoblasts consisting mainly of basophile types.

Among the other cells yielded by sternal puncture of normal material are a few megacaryocytes and giant cells, some old,





others young. There is always a certain percentage of eosinophile leucocytes, mainly young forms. There is always a small percentage of myeloblasts, a slightly greater number of praemyelocytes, and comparatively numerous neutrophile myelocytes. The cell always present in the greatest quantities is the neutrophile metamyelocyte (about 30 per cent.). A smaller percentage is represented by neutrophile band forms and polymorphs. The monocytes are comparatively rare, whereas the lymphocytes are quite numerous (10 to 20 per cent.). As a rule, a few reticulo-endothelial cells are to be seen, and there are always some macerated cells.

The nomenclature and methods of calculation in the literature are so varied that it is practically impossible to undertake a detailed comparison with my own material of differential counts from sternal puncture of normal cases. One thing is at any rate certain: The findings must be fairly alike considering the above-mentioned similarity of the results with regard to the numbers of the nucleated blood cells per  $\text{mm}^3$ , in sternal blood and the relationship between the erythropoietic and the leucopoietic cells.

Table 3 gives a summary of the findings of repeated sternal puncture of my untreated cases of pernicious anaemia. Note that the variations in the differential counts from one and the same patient are remarkably small.

My own observations and those of other workers referred to in this chapter show that sternal puncture is well suited to the investigations on which this work is based.

#### *Conclusion:*

1) *The results obtained by several observers and by myself are in agreement with regard to the qualitative and quantitative composition of normal sternal marrow.*

2) *My own investigations of sternal puncture repeated several times on the same patient have yielded uniform findings.*

3) *The average number of nucleated blood cells from sternal puncture is 100,000 per  $\text{mm}^3$ , a range of variation from 20,000 to fully 200,000.*

4) *The average percentage of erythroblasts in relation to the nucleated blood cells from sternal puncture is between 15 and 20, with a range of variation from 10 to 25 per cent.*



5) *On rare occasions practically pure foci of fat are found in normal sternal marrow.*

6) *The average incidence of other nucleated cell forms found on sternal puncture is mentioned.*

7) *Sternal puncture in normal persons is associated with a moderate degree of aspiration pain.*

8) *Normal sternal puncture yields numerous macroscopic particles of marrow and fat, the latter being somewhat the more numerous of the two.*

9) *With the technique employed, sternal puncture gives a reliable picture, quantitative and qualitative, of the nucleated blood cells of the sternal marrow.*

## VI. The Sternal Marrow in Untreated Pernicious Anaemia.

### A. Quantitative conditions.

1) *Previous investigations.* Ever since COHNHEIM in 1876 concluded from the extensive distribution of red bone marrow in pernicious anaemia that it is a disease of the bone marrow, the opinion has been held that there is a great increase of red, pathological bone marrow. The microscopic picture has been taken to indicate a profusion of the precursors of the erythrocytes, but the character of these precursors has been in dispute.

Most observers have subsequently regarded the bone marrow as functionally hyperactive and hyperplastic. It was held that on account of the great destruction of cells supposed to be due to phagocytosis or haemolysis, the bone marrow was unable to create enough blood cells to balance this loss.

In 1926, MINOT and MURPHY introduced liver treatment which created new arguments in the discussion of haemopoiesis in pernicious anaemia. Where and how did the antipernicious anaemia factor act?

Many still believe that pernicious anaemia is due to increased blood destruction, witness the high bilirubin figures in the blood and the excretion to some extent of large quantities of urobilinogen in the urine. This excretion was supposed to proceed parallel with the progress of the anaemia and to disappear with liver treatment whose introduction has brought much to the fore the theory that inhibition of the bone marrow's function is due to failure of the supply of the antipernicious anaemia factor. How this factor is related to erythropoiesis is not yet quite clear. Many hold that some constituent of the liver «loosens the bonds» preventing the bone marrow from reacting to the stimulus of pernicious anaemia, or

that the youngest precursors of the erythrocytes accumulate because of an insufficiency of the supply of the antipernicious anaemia factor necessary for their ripening. When this factor is supplied, the bone marrow again becomes normal. WHIPPLE sees in the large quantities of bilirubin and urobilinogen a stimulus to blood formation, not as indicating any kind of destruction, but the presence of unemployed pigment.

On the background of these observations, very briefly referred to, we must try to interpret the sternal marrow findings in pernicious anaemia and ask ourselves if there are not other possible explanations of the state of the marrow in this disease.

In the literature there are only a few and scattered isolated observations on the number of the nucleated blood cells in the marrow in pernicious anaemia.

ELSA SEGERDAHL has found an average of 129,100 ( $\pm$  18,400) nucleated blood cells per mm.<sup>3</sup> from the sternal puncture of 24 cases of untreated pernicious anaemia. In 1935, YOUNG and OSGOOD examined four cases of untreated pernicious anaemia (anaemia from 1.2 to 1.89 million erythrocytes) and found the erythroblasts from sternal puncture to represent from 32.3 to 57.1 per cent. of the nucleated blood cells. ZANATY examined seven patients (anaemia from 1.36 to 3.76 million erythrocytes) and found the percentage of erythroblasts to range from 38 to 75.

All agree that the bone marrow of pernicious anaemia is abnormally rich in cells, but figures are seldom given. On these rare occasions, the figures are put at 200,000 to 300,000 cells per mm. (SCHÜLTEN, HENNING and KEILHACK, ROHR, SCHILLING, KLIMA, SEGERDAHL, MARKOFF, PENATI, MALARMÉ, P. E. WEIL, THADDEA, BAKALOS and many others).

*Own investigations.* My own material consists of 49 cases of untreated pernicious anaemia in which 71 sternal punctures were made. The figures are not quite comparable as from 0.1 to 1 cc. of sternal blood was aspirated after three to five smears had been prepared.

As table 4 shows, the nucleated blood cells ranged from 5,000 to 240,000 per mm. These figures must be regarded as the extreme limits to be discussed later on; On the whole, these figures lay very evenly around the average figures in this table.

Table 4. The number of nucleated blood cells found on sternal puncture and the percentage of erythroblasts among them in untreated cases of pernicious anaemia. Arrangement according to the degree of the anaemia. Own material.

Case No	Erythrocytes in mill. Capillary blood	Erythroblasts per cent of the nucleated sternal marrow cells	Nucleated sternal cells. per mm <sup>3</sup>	Number of punctures
11	0.71	26.5	5,000	Marrow atrophy
4	0.89	35.5	61,800	1
54	0.89	39.8	128,000	1
50	0.91	56.8	112,500	2
56	0.94	54.8	137,800	1
36	0.95	48.5	64,200	1
25	1.01	37.5	101,500	1
18 b	1.11	55.2	226,300	1
10	1.12	47.6	71,000	1
59	1.18	33.3	49,200	1
14	1.19	38.0	74,800	1
30	1.20	30.2	—	1
26	1.22	35.6	68,100	5
22	1.26	35.1	78,600	1
52	1.28	47.2	62,600	3
33	1.33	50.9	208,100	1
Average	1.10 million	43.7 per cent	103,200	
47	1.33	33.5	100,000	3
18 a	1.37	40.4	64,300	1
1	1.39	27.0	87,000	1
57	1.40	41.7	93,200	1
28	1.42	52.7	78,400	3
32	1.42	35.0	98,000	1
58	1.43	53.9	57,900	1
6	1.44	46.4	34,500	4
51	1.48	41.4	51,300	2
53	1.50	44.9	118,500	3
2	1.53	45.7	51,000	1
27	1.58	22.0	—	1
23	1.67	32.9	72,900	1
48	1.75	40.5	140,800	2
Average	1.48 million	39.9 per cent	80,600	
7	1.82	34.2	44,700	2
24	1.88	34.2	79,800	1
49	1.89	42.0	77,200	2
8	1.95	46.9	50,000	1
34	1.95	57.8	77,600	1
19	1.96	35.0	191,900	1
5	2.00;;	27.3	54,600	3
55	2.01	33.3	—	1
13	2.04	34.6	123,700	1
16	2.06	22.9	66,000	1
12	2.17	26.8	21,800	1
Average	1.98 million	35.9 per cent	78,700	
35	2.27	23.6	42,100	1
17	2.37	30.4	64,800	1
15	2.38	24.0	44,900	1
31	2.38	29.0	9,100	1
29	2.46	61.3	240,000	1
37	2.69	32.0	104,800	1
21	2.87	23.9	42,950	1
20	3.15	16.0	30,900	1
9	3.61	18.8	38,700	1
Average	2.69 million	28.8 per cent	68,700	

The lowest figure (5,000) is much below the lowest limit for normal punctures. Several features in the record of this case throw light on this low figure. Numerous particles of fat and no particles of marrow were aspirated. The relative admixture of capillary blood was also great. The differential count showed a comparatively high figure for polymorphs, and very few young forms. Evidently in this case the marrow aspirated was rich in fat and was mixed with much capillary blood. As, however, 26.5 per cent. of the cells consisted of erythroblasts, there must have been some erythropoietic tissue in the area aspirated. When sternal puncture was repeated 14 hours later in the same area, no fat particles, but a certain number of marrow particles were found. There were 43,300 nucleated blood cells per mm.<sup>3</sup> in spite of the fact that a quite considerable quantity of sternal blood (0.55 cc.) was aspirated. It should, however, be noted that the patient had received an injection of liver extract just after the first sternal puncture. But the differential count of the sternal blood gave no ground for thinking that this treatment had had any demonstrable effect after so short an interval, for the morphology of the cells was identical with what is usually found in untreated pernicious anaemia. This case would thus seem to show that, in pernicious anaemia, foci of fat may be found interspersed in the red bone marrow. The patient's peripheral blood contained 0.68 million erythrocytes per mm. Here it is conceivable that the foci of fat were indicative of disappearance of red bone marrow which it displaced. As already pointed out, similar foci of fat have been found post mortem in cases of pernicious anaemia.

In 1910, ZIEGLER found in «pernicious anaemia» several cases of aplasia of the red bone marrow, and DAMESHEK and VALENTINE, who examined 20 patients with Seyfarth's trephine, claimed to have found «a complete replacement of fat by proliferating cells.» ROHR has never found «hypoplastic or aplastic» sternal marrow in pernicious anaemia.

This table also shows that in case 31, sternal puncture yielded few nucleated blood cells. In this case, also, much fat was aspirated, and the same reflections are applicable to this as to the previous case. Under liver treatment the sternal marrow and the patient's anaemia reacted most satisfactorily. There were 2.38 million

erythrocytes in the peripheral blood of this patient. Thus we may expect to find the sternal marrow containing quite a quantity of persisting fatty tissue here and there during the progress of the anaemia. One often finds numerous particles of fat on sternal puncture from time to time in cases in which at other times a plentiful supply of marrow particles and nucleated blood cells is noted. During the reaction of the sternal marrow to liver treatment in the primary hyperplastic phase, sternal puncture hardly ever yields fat particles. In moderately severe pernicious anaemia, the fatty tissue found must be regarded as a remnant of the fatty tissue in normal sternal marrow.

With regard to the highest figures in the table, we may note that in case 29 there were 240,000 nucleated blood cells per mm.<sup>3</sup>, and that, on a differential count, 61.3 per cent. of them were erythroblasts. There were 2.46 million erythrocytes in the peripheral blood, and from earlier observations one would not expect to find a very high count of nucleated blood cells on sternal puncture and the number of erythrocytes in the peripheral blood to be very high in so moderate a degree of anaemia. Otherwise there was nothing remarkable about the patient whose reaction to liver treatment was exceptionally good. After five days the reticulocytes had risen to 179<sup>0</sup>/<sub>100</sub> which is a very high figure for so moderate a degree of anaemia. The nucleated blood cells also increased in a very satisfactory way, reaching to over 400,000 two days after the injection. Evidently the functional activity of the bone marrow in this case was unusually lively, witness also the comparatively high figures noted. Each time three punctures were made in the course of five days, very severe aspiration pain was felt, and while there was no yield of fat, the marrow particles were numerous, — observations pointing in the same direction.

Cases 33 and 19 may be interpreted in the same way. It is possible that in case 19 an area exceptionally rich in cells was encountered, for at five subsequent punctures the counts of the nucleated blood cells were invariably low, to some extent very low, even after liver treatment.

The figures for the other cases are more even, and indeed so even that they can serve for a calculation of averages which, when the above-mentioned reservations are taken with regard to tech-

nique and interpretation of bone marrow findings, may serve as a basis for the study of the bone marrow and the part it plays in pernicious anaemia.

Table 4, which is arranged according to increasing degrees of anaemia, shows that, on the average, the number of nucleated cells obtained by sternal puncture rises as the anaemia progresses. Departures from this trend are referred to. The table is divided into four parts, without any definite criteria for their limits, and the averages for each are calculated. Here we see the average number of the nucleated cells rise from 68,700 to 103,200 per mm. as the anaemia rises from moderate to severe. The average for all these parts is about 80,000. All the figures lie within the limits of the findings for normal sternal puncture, and the average of 80,000 is almost 20,000 nucleated blood cells per mm. less than the normal. Table 4 also shows that the factor contributing most to the rise from the comparatively small figures in moderate anaemia to the slightly larger figures in more severe anaemia is the increase in the number of erythroblasts. As the table shows, the comparative number of erythroblasts rises evenly with the development of the anaemia, from the somewhat raised percentage of 28.8 in moderate anaemia to the very high percentage of 43.7 in marked anaemia. In the light of the small number of nucleated blood cells, these figures tell us that the absolute number of erythroblasts shows a very doubtful rise if it can be called a rise at all, and a reduction in the number of cells of the leucocyte types.

Table 5 is framed so as to show the rising percentage of erythroblasts of sternal marrow. Here we see how these percentages and the number of nucleated blood cells yielded by sternal puncture rise as the anaemia progresses. Like table 4 this table is divided into four parts on the same principle as before. It shows the same things, but in another way. As the percentage of erythroblasts rises and the cells of the leucocyte types in the sternal marrow become fewer, we find a growing number of nucleated blood cells on sternal puncture and increasing anaemia.

Table 6, which is based on a study by I. BARTA of a small material, is drawn up for comparison with my own findings. This table shows that BARTA has found the same conditions with regard to the percentage of erythroblasts, with this exception that his figures are considerably higher.

*Table 5.* The number of nucleated blood cells found on sternal puncture and the number of erythrocytes in the peripheral blood. Arrangement according to the rising percentage of erythroblasts among the nucleated blood cells found on the sternal puncture of patients with untreated pernicious anaemia. Own material.

Case No.	Erythroblasts per cent of the nucleated cells in the sternal marrow	Erythrocytes in mill. Capillary blood	Nucleated cells in sternal marrow per mm <sup>3</sup>
29	61.3	2.46	210,000
34	57.8	1.95	77,600
50	56.8	0.91	112,500
18 b	55.2	1.11	226,300
56	54.8	0.91	137,000
58	53.9	1.43	57,900
28	52.7	1.42	78,400
33	50.9	1.33	208,100
36	48.5	0.95	61,200
10	47.6	1.12	71,000
52	47.2	1.28	62,600
8	46.9	1.95	50,000
6	46.4	1.44	31,500
2	45.7	1.53	51,000
53	44.9	1.50	118,500
Average	51.4 per cent	1.42 million	106,000
49	42.0	1.89	77,200
57	41.7	1.40	93,200
51	41.4	1.48	51,300
48	40.5	1.75	140,000
18	40.4	1.37	61,300
54	39.8	0.89	128,000
14	38.0	1.19	74,000
25	37.5	1.01	101,500
26	35.6	1.22	68,100
4	35.5	0.98	61,800
22	35.1	1.26	78,600
19	35.0	1.96	191,000
32	35.0	1.42	98,000
Average	38.3 per cent	1.37 million	91,400
24	34.6	2.04	123,700
7	34.2	1.82	44,700
13	34.2	1.88	79,800
47	33.5	1.33	100,000
55	33.3	2.01	—
59	33.3	1.18	49,200
23	32.9	1.67	72,900
37	32.0	2.69	104,800
17	30.4	2.37	61,800
30	30.2	1.20	—
31	29.0	2.38	9,100
5	27.3	2.00	51,600
Average	32.1 per cent	1.88 million	70,400
1	27.0	1.39	87,800
12	26.8	2.17	21,800
15	24.0	2.38	44,900
21	23.9	2.87	42,950
35	23.6	2.27	42,100
16	22.9	2.06	66,000
27	22.0	1.58	—
9	18.8	3.61	38,700
20	16.0	3.15	30,900
Average	22.7 per cent	2.39 million	46,900



Table 6. The incidence per cent. of erythroblasts and megaloblasts on sternal puncture in pernicious anaemia, based on I. Barta's study.

Erythrocytes in the peripheral blood in millions	Percentage of erythroblasts among the nucleated blood cells of the sternal marrow	Percentage of megaloblasts among the erythroblasts of the sternal blood
0.84	77	56
0.98	73	51
1.04	71	48
1.70	68	31
1.85	62	27
2.80	51	4
3.29	49	0
3.54	46	0
3.98	45	0
4.10	41	0

As the case records show, sternal puncture gave rise to much more pain than is experienced by normal persons. We must accept as a well established fact the observation that the bone marrow in untreated cases of pernicious anaemia is much more sensitive to aspiration than in health. In my experience this is also the case in *genuine* hyperplastic conditions such as congenital haemolytic jaundice, whereas under hypoplastic conditions aspiration is practically painless.

**Conclusion:** When pernicious anaemia is not treated, sternal puncture yields:

- 1) *A relatively moderate number of nucleated blood cells per mm.<sup>3</sup>*
- 2) *They show a rising tendency as the anaemia progresses, their numbers being within the normal limits, the average being under the normal average.*
- 3) *The percentage of erythroblasts is raised.*
- 4) *This rise attains very high figures as the anaemia progresses.*
- 5) *It seems to be a slight rise in the absolute number of erythroblasts, and a fall in the number of cells of the leucocyte types.*
- 6) *All the time throughout the development of pernicious anaemia, foci of fat may be found in the sternum.*
- 7) *But as a rule, particles of marrow become more numerous and particles of fat less numerous as the anaemia progresses.*

8) *Agonal disappearance of the red bone marrow is a possibility to be considered.*

9) *In certain cases we must expect to find the sternal marrow functioning in a more lively way than is usual in pernicious anaemia.*

10) *Aspiration of sternal blood is associated with pain definitely greater than under normal conditions.*

## B. Quantitative Conditions.

1) *Previous investigations.* Ever since investigation of the bone marrow in pernicious anaemia was started, attention has been drawn to the great number of mononuclear cells which for a long time were thought to present a lymphoid character (NAEGELI, ZIEGLER). They were called lymphoidocytes by PAPPENHEIM and erythrocytes by HELLY. In 1924, V. SCHILLING took the definite standpoint that the «lymphoid marrow» of pernicious anaemia is of a erythropoietic character.

As already repeatedly pointed out in this work, a discussion of this subject encounters difficulties of nomenclature. One fails to find in the literature accounts of investigations — at any rate on a large scale — in which, by differential counts of sternal marrow smears, an attempt has been made to ascertain the interrelationship of all the various types of erythroblast during the development of pernicious anaemia. Yet there are many works in which this problem is discussed, and according to most of them there is a profusion of megaloblasts (LEITNER, COTTI and CIBOLDI, BRUGSCH, WINTROBE, ELLERMANN, HENNING, MERWE, SEGERDAHL, BARTA, ZIEGLER and many others).

With reference to the purely numerical conditions, GIOVANI GHEDINI in 1910 found on puncture of the tibia of a man aged 57 already referred to (he may possibly have had pernicious anaemia) for every 100 erythrocytes obtained by tibia trephine, 15 «megaloblasts» and 47 «normoblasts», as well as numerous divisional forms. This method of calculation and estimate of morphology does not give even approximately useful results with which to compare present day investigations. YOUNG and OSGOOD (1935) have found a rise in the number of «megaloblasts» and «normoblasts». MARKOFF and STORTI (1937) as well as TEMPKA and BRAUN have done so too.

In 1921, ZADEK made a similar finding on trephining the tibia, and BARTA on trephining the sternum. ZANATY found »megaloblasts, macroblasts and normoblasts», and his »megaloblasts» constituted on the average 40 to 50 per cent, of the cells of these types in untreated cases of pernicious anaemia with from 1.36 to 2.13 million erythrocytes in the peripheral blood (seven cases). SCHULTEN, who found many »proerythroblasts» made a similar finding. TEMPKA and BRAUN (1932) found proerythroblasts, normoblasts and megaloblasts, while DAMESHEK and VALENTINE found great numbers of erythrogenies (»promegaloblasts»), but very few more mature forms. THADDEA and BAKALOS, PEABODY, SEGERDAHL, PENATI and SAITA as well as many others have made and described similar findings.

A collective survey of the findings of all these authors shows that they agree over the number of the erythroblasts, particularly the younger forms, being increased. As the nomenclature differs greatly according to the haematological school to which various observers belong, and as their conception of erythropoiesis lacks uniformity, the literature concerning the morphology of the constituents of the bone marrow in pernicious anaemia presents a very confused picture.

*Own investigations.* The sternal marrow in 50 cases of untreated pernicious anaemia was examined by differential counts of cells yielded by 71 punctures made at various stages in the development of the untreated disease. There should therefore be sufficient material by which to form an estimate of the morphological development of the sternal marrow in the progressive course of the disease. In this investigation special attention was paid to erythropoiesis.

Tables 7, 8, 9 and 10 give the differential counts from smears obtained by sternal puncture, only the result of one puncture being noted in the cases in which several punctures were made before treatment with liver extract was instituted. Table 3 gives the results of several punctures of the marrow of one and the same patient before treatment, and, as already pointed out, these figures corroborate one another. The tables are arranged so that the findings of the differential counts follow the development of the pernicious anaemia. Table 7 begins with the findings in cases with 0.71

Table 7. Differential count of sternal puncture smears from untreated cases of pernicious anaemia with 0.71 to 0.95 million erythrocytes in the peripheral blood. Owa material.

Cases:				11	4	54	50	56	36
Erythropoiesis	Pro- megalobl. macrobl.	per cent		—	7.50	3.—	5.25	18.—	22.50
	Megalobl.	bas.	"	5.70	5.50	10.—	11.25	2.—	2.—
		eos.	"	—	—	—	—	—	—
	Macrobl.	bas.	"	13.20	66.50	59.50	58.25	15.—	60.00
		eos.	"	11.30	15.—	3.—	22.25	41.50	8.—
	Normobl.	bas.	"	—	—	—	—	—	—
		eos.	"	—	—	—	—	—	—
	Erythrbl.	bas.	"	19.30	0.50	12.50	0.25	5.50	4.50
		eos.	"	50.50	4.50	10.50	0.50	16.—	—
	Erythrbl.	bas.	"	—	0.50	1.50	1.75	1.—	3.—
Leucopoiesis	division forms	eos.	"	—	—	—	0.50	1.—	—
	Megacaryocytes	"	"	—	0.25	0.25	0.25	0.25	—
	Mast immature	"	"	—	0.25	0.25	0.75	—	0.25
	cells mature	"	"	—	—	—	—	—	0.25
	Eos. Myelocytes	"	"	—	2.25	0.75	1.50	1.00	3.50
	Leuco- band forms	"	"	1.—	2.75	0.50	1.75	2.—	1.50
	cytes polymorphs	"	"	1.50	3.—	0.50	0.25	0.25	2.50
	Myeloblasts	"	"	—	6.75	1.75	3.50	2.—	2.50
	Praemyelocytes	"	"	3.—	7.50	7.50	6.25	11.—	3.75
	Neu- myelocytes	"	"	5.—	19.25	16.25	19.25	16.50	18.—
	tro- young forms	"	"	7.50	26.75	50.75	26.50	36.75	37.—
	philes band forms	"	"	11.50	4.75	8.50	13.—	6.50	2.50
	philes polymorphs	"	"	35.—	3.25	6.25	5.75	5.—	6.—
	Mono- blasts	"	"	—	—	—	—	—	—
	cytes	"	"	1.—	0.25	—	0.25	—	1.25
	Lymphocytes	"	"	20.50	11.25	1.—	4.—	3.75	2.25
	Plasma & Türk cells	"	"	—	0.25	1.25	2.—	2.25	—
	Smear cells	"	"	14.—	11.25	3.25	10.75	9.25	17.50
	Reticuloendothelial cells	"	"	—	0.25	1.25	4.25	3.50	1.75

million erythrocytes per mm<sup>3</sup>. in the peripheral blood, and table 10 ends with the findings in cases with 3.61 million erythrocytes. I have also collected in groups the findings in cases in which the erythrocyte counts were within half a million of each other, and I have calculated the averages so as to ascertain if any numerical index could be found to the changes taking place during the development of the anaemia (table 13).

Table 8. Differential count of sternal puncture smears from untreated cases of blood. Own

Case:			25	18 b	10	59	14	30	26	22
Erythropoiesis	Pro- megalobl. per cent	macrobl.	4.50	11.—	3.—	6.50	—	2.—	5.50	21.—
	Megalobl.	bas. *	9.50	5.—	3.50	13.50	10.—	15.—	11.50	14.50
		eos. "	—	—	—	—	1.60	—	—	2.—
	Macrobl.	bas. "	58.50	59.—	31.50	53.50	33.70	46.—	75.—	52.50
		eos. "	9.—	18.—	24.50	8.50	6.80	29.50	1.50	6.—
	Normobl.	bas. *	—	—	—	—	—	—	—	—
		eos. "	—	—	—	—	—	—	—	—
	Erythrobl.	bas. "	3.—	1.—	2.—	4.—	22.60	0.50	0.50	1.—
		eos. "	13.—	5.50	35.—	14.—	24.20	5.50	2.50	2.50
	Erythrobl.	bas. "	2.—	0.50	—	—	1.10	1.50	3.50	0.50
Leucopoiesis	division forms	cos. "	0.50	—	1.—	—	—	—	—	—
	Megacaryocytes	"	—	0.20	—	—	—	—	—	0.25
	Mast immature	"	0.50	—	0.50	—	0.25	0.25	—	—
	cells mature	"	0.25	—	0.25	—	—	—	—	—
	Eos. myelocytes	"	1.75	2.70	0.50	3.25	0.75	0.25	1.75	3.50
	Leu- band forms	"	1.75	1.80	0.50	2.75	0.25	0.25	2.75	2.25
	cocyt. polymorphs	"	0.50	2.50	—	1.—	0.75	2.—	1.25	2.25
	Myeloblasts	"	6.—	2.30	1.25	1.—	1.25	2.50	1.25	3.75
	Praemyelocytes	"	5.75	7.70	6.25	5.75	2.25	2.75	3.25	5.75
	Ncu- myelocytes	"	17.25	14.50	12.—	10.25	18.75	10.25	19.50	15.75
	tro- yong forms	"	25.25	38.—	32.—	21.25	20.—	28.50	29.—	35.75
	philes band forms	"	7.75	7.70	5.50	4.75	21.—	10.75	12.25	7.25
	polymorphs	"	6.50	4.80	4.75	5.—	9.75	10.75	11.75	7.25
	Mono- blasts	"	0.50	—	—	—	0.50	2.—	—	1.25
	cytes	"	0.50	—	—	—	0.50	2.—	—	1.25
	Lymphocytes	"	13.50	6.10	14.50	25.50	14.75	18.50	2.75	4.25
	Plasma & Türk cells	"	0.50	—	0.50	—	—	—	—	1.25
	Smear cells	"	12.—	12.60	21.50	14.50	9.50	10.25	13.50	4.—
	Reticuloendothel. cells	"	0.25	1.10	—	5.—	0.25	1.—	1.—	5.—

As the tables show, the development of erythropoiesis in the sternal marrow in pernicious anaemia, from its onset to its complete development, falls according to the morphological findings into three different stages.

1. The normo-macroblastic stage.
2. The normo-macromegaloblastic stage.
3. The macromegaloblastic stage.

To judge by the tables, most of the patients I have examined

pernicious anaemia with 1.01 to 1.48 million erythrocytes in the peripheral material.

52	33	47	18 a	1	57	28	32	58	6	51
18.—	15.—	4.—	11.—	1.—	6.—	4.—	—	11.—	1.—	8.—
13.—	10.50	5.—	3.40	2.—	16.50	15.—	16.40	32.50	8.20	15.—
—	—	—	3.—	2.—	—	1.—	3.—	—	—	—
39.—	65.50	53.—	22.80	53.—	59.—	44.50	30.—	44.50	38.—	43.—
1.—	8.—	23.—	10.40	26.—	8.—	3.—	—	14.50	3.80	8.—
—	—	—	—	—	—	—	—	—	—	—
—	—	—	1.—	—	—	—	—	—	—	—
17.50	—	1.50	17.80	—	3.50	31.50	48.60	1.50	38.40	13.—
10.—	1.—	9.50	29.60	14.—	4.—	0.50	—	2.50	11.10	10.—
1.50	—	1.50	—	2.—	1.—	0.50	—	1.—	0.50	2.50
—	—	2.50	1.—	—	2.—	—	—	1.50	—	0.50
—	—	0.25	—	—	—	—	0.25	—	—	—
1.25	—	—	0.25	—	0.50	1.—	—	0.25	0.25	0.50
—	—	—	—	—	—	—	—	0.25	—	—
2.75	0.25	—	1.25	1.75	4.25	2.—	3.25	1.25	1.50	1.75
4.75	2.50	—	0.75	2.75	1.75	3.—	2.—	0.25	2.25	1.—
4.75	0.75	1.75	1.—	0.75	2.25	1.—	1.—	1.—	0.75	0.75
1.75	3.25	2.25	1.50	3.—	1.25	2.50	1.50	2.50	1.—	1.75
7.75	3.—	1.50	4.25	4.—	3.—	4.50	8.75	4.75	2.25	6.—
9.50	19.50	12.50	17.—	16.50	32.25	15.50	9.—	26.—	18.25	18.50
32.50	39.—	17.50	26.75	39.50	37.—	32.50	9.75	46.25	24.—	30.75
8.25	9.75	7.25	15.25	2.25	4.75	5.50	28.25	4.25	18.—	12.50
11.50	5.50	27.—	11.75	8.50	2.50	6.50	25.—	4.25	8.—	12.50
—	—	1.75	—	0.25	0.25	—	2.25	—	1.75	—
7.25	3.—	14.25	9.75	4.25	2.50	5.50	8.—	3.25	13.—	7.50
—	—	0.50	0.75	—	0.75	—	0.25	0.50	—	0.50
5.25	11.—	9.75	9.75	14.75	6.—	20.50	—	4.50	8.25	4.75
2.75	2.—	3.75	—	1.75	1.—	0.50	0.75	0.50	0.75	1.25

would seem to belong to the third stage, and this was to be expected as the symptoms seldom bring patient to doctor at an earlier stage.

Tables 11 and 12 are arranged on the same principle adopted in the earlier tables, and they show the findings of erythropoiesis during the development of pernicious anaemia. In the first group the promacromegaloblasts and the megaloblasts are collected together, with the megaloblasts by themselves as a sub-group. The

Table 2. Differential count of sternal puncture smears from untreated cases of pernicious anaemia with 1.50 to 1.96 million erythrocytes in the peripheral blood. Own material.

Case:	53	2	27	23	48	7	24	49	8	34	19
Pro- megabl. macrobl.	per cent										
Megalobl.	7.50	—	6.—	18.50	13.70	11.50	6.50	11.50	8.50	7.—	9.—
bas.	17.—	0.50	10.—	8.50	3.70	10.50	5.—	9.50	7.50	10.30	10.40
eos.	—	0.50	—	—	—	—	—	—	—	3.70	1.70
Macrobl.	30.—	62.25	36.—	52.—	51.30	78.—	75.—	58.50	65.—	16.70	33.20
bas.	35.—	—	25.—	7.—	20.—	—	9.50	11.—	8.50	2.—	—
Normobl.	—	—	—	—	—	—	—	—	1.—	—	—
bas.	—	—	—	—	—	—	—	—	1.—	—	0.50
Erythrobl.	—	23.75	10.—	4.—	1.—	—	—	0.50	1.—	58.70	45.20
bas.	9.—	12.75	10.—	6.50	18.70	—	1.—	4.50	6.50	—	—
bas.	0.50	0.25	3.—	3.50	0.30	—	2.—	3.—	1.—	1.60	—
division forms	1.—	—	—	—	0.30	—	1.—	0.50	—	—	—
Megacaryocytes	—	—	—	—	—	—	—	—	—	0.25	—
Mast cells	0.75	—	0.25	0.75	0.75	—	0.25	0.25	0.25	—	—
immature	—	—	—	0.25	—	—	—	—	—	0.25	—
mature	—	—	—	0.25	—	—	—	—	—	—	—
Eos.	3.25	0.25	0.25	0.75	0.50	0.50	1.—	2.—	1.50	1.75	2.50
myelocytes	3.50	1.25	2.—	1.25	1.—	2.75	2.50	1.75	0.50	2.—	2.75
band forms	1.50	0.75	1.75	0.25	0.25	1.—	0.75	2.25	1.25	2.25	3.—
polymorphs	2.—	1.—	1.25	2.25	5.50	1.50	0.50	3.—	4.—	1.—	1.25
Myeloblasts	8.25	5.50	1.50	1.50	4.25	6.50	11.—	2.50	5.75	11.25	1.75
Praemyelocytes	13.25	8.—	10.25	13.25	18.25	21.50	19.75	12.50	19.25	7.75	13.75
Neutrophils	31.—	32.25	21.50	26.50	26.25	34.75	31.25	18.75	20.75	28.75	18.75
myelocytes	5.—	23.—	14.75	15.—	9.—	10.75	9.25	6.75	8.50	19.50	40.—
young forms	2.25	27.50	30.75	15.75	8.75	8.25	5.25	11.75	4.25	17.50	11.—
band forms	—	—	—	—	—	—	—	—	—	—	—
polymorphs	1.25	—	1.75	0.50	—	0.75	—	2.25	0.75	1.25	—
blasts	—	—	—	—	—	—	—	—	—	—	—
cytes	13.50	0.50	10.—	10.25	13.75	5.—	2.—	15.25	13.75	5.—	1.50
Lymphocytes	0.50	—	—	0.50	0.25	—	—	0.75	1.—	—	—
Plasma & Türk cells	9.25	—	3.50	11.25	3.—	6.75	16.50	12.—	17.—	—	0.75
Smear cells	4.75	—	—	0.25	8.75	—	—	8.25	1.25	0.50	2.—
Retendothelial cells	—	—	—	—	—	—	—	—	—	—	—

cases of pernicious anaemia with from 2.00 to 3.81 million.

Table 10. Differential count of sternal puncture smears from untreated cases of pernicious anaemia with from 2.00 to 3.61 million erythrocytes in the peripheral blood. Own material.

Cases:		5	55	13	16	12	35	17	15	31	29	37	21	20	9
Erythropoiesis	Pro- megalobl. per cent	—	4.—	31.70	—	3.50	10.—	0.50	—	6.—	4.—	0.50	4.—	4.—	—
	Megalobl. bas.	13.—	13.50	9.20	5.—	12.—	16.30	3.50	2.60	—	15.50	5.70	2.—	5.—	—
	cos.	—	—	—	—	—	3.50	—	—	—	1.—	5.70	1.—	—	—
	Macrobl. bas.	46.10	51.—	13.30	62.—	51.50	28.—	21.—	13.50	32.—	44.—	46.30	51.—	57.—	20.—
	cos.	5.20	12.—	—	4.—	23.—	2.50	2.50	1.70	7.—	3.—	—	7.—	14.—	3.—
	Normobl. bas.	3.50	—	—	10.—	—	7.60	62.—	26.20	40.—	—	—	17.—	1.—	56.—
	cos.	—	—	—	7.—	—	—	6.—	17.80	5.—	—	6.80	8.—	12.—	20.—
	Erythrobl. bas.	13.90	11.50	44.60	2.—	2.50	17.—	—	27.90	7.—	31.50	7.30	7.—	5.—	1.—
	cos.	15.70	7.50	—	5.—	7.50	12.70	1.50	9.40	2.—	0.50	12.—	3.—	2.—	—
	division forms cos.	2.60	0.50	1.20	5.—	—	2.50	2.50	0.90	1.—	0.50	6.30	—	—	—
Leucopoiesis	division forms cos.	—	—	—	—	—	—	0.50	—	—	—	—	—	+	—
	Megacaryocytes	—	—	—	—	0.50	0.25	0.50	—	—	—	—	—	—	—
	Mast immature cells	—	0.75	—	0.20	0.50	0.25	0.25	0.50	0.50	1.—	0.50	0.25	0.25	0.25
	mature	—	—	—	—	—	0.25	—	—	—	—	—	—	0.50	—
	Eos. myelocytes	1.25	1.75	3.50	1.20	0.50	0.25	0.75	3.25	—	2.—	3.—	0.75	1.75	0.75
	leuco- band forms	0.75	1.75	1.75	1.—	1.—	3.50	2.25	2.—	0.50	3.—	2.25	0.75	1.50	1.75
	cytes polymorphs	1.—	1.75	1.50	1.—	0.50	1.75	1.75	1.25	—	1.—	1.75	1.—	2.50	0.50
	Myeloblasts	2.—	2.25	0.25	2.70	4.25	0.50	1.25	2.75	1.—	2.50	2.25	2.—	3.75	0.25
	Praemyelocytes	5.—	4.75	3.—	4.—	4.25	6.25	2.50	2.75	2.50	4.50	2.75	2.—	3.75	4.—
	Neu- myelocytes	10.25	11.25	19.—	15.20	16.75	8.—	16.75	17.75	6.50	15.50	7.25	11.75	15.25	22.25
	tro- young forms	16.50	26.50	32.50	22.50	22.50	19.—	36.—	23.75	9.—	32.50	24.75	18.50	27.—	40.25
	philes band forms	12.50	6.—	25.—	14.—	9.—	15.25	14.50	17.25	7.50	6.50	26.50	12.50	4.25	14.50
	polymorphs	25.50	11.25	6.50	15.—	19.75	27.75	14.—	14.25	35.—	5.50	21.—	30.50	28.25	6.50
	Mono- blasts	}		—	—	2.25	0.25	2.—	0.50	1.50	—	0.75	0.50	1.50	0.50
	cytes			—	1.20	—	—	—	—	—	—	—	—	—	—
	Lymphocytes	14.50	18.25	1.—	13.20	6.—	11.—	5.75	8.75	21.—	5.50	6.—	11.50	2.75	0.75
	Plasma & Türk cells	0.25	0.25	0.25	—	0.25	0.50	0.25	0.75	—	—	—	0.50	0.25	0.50
	Smears cells	9.50	12.75	—	8.20	10.50	4.—	1.50	4.50	15.—	20.—	—	7.50	6.25	7.—
	Reticuloendothel. cells	—	0.75	4.50	0.70	1.50	1.25	—	—	—	0.50	—	—	0.50	0.25



Table 11. Percentage interrelationship of the various types of erythroblast from sternal puncture smears in untreated cases of pernicious anaemia. Arrangement according to the degree of the anaemia in each case, and the average erythroblasts counts within a range of half a million erythrocytes per m.m.<sup>3</sup> in the peripheral blood. In group 1, the percentage of megaloblasts is put by itself beside the first column, and in group 2 the mitosis percentage by itself beside the first column. Own material.

Case No.	Promakro-megalo- blasts and megaloblasts in Sternal marrow per cent.		Makroblasts, ery- throblasts and ery- throblasts in mitosis per cent		Normo- blasts per cent	Erythrocytes in blood capil- lary in mil- lions
11	5.7	5.7	94.3	0.0	0	0.71
4	13.0	5.5	87.0	0.5	0	0.89
54	13.0	10.0	87.0	1.5	0	0.89
50	16.5	11.25	83.5	2.25	0	0.91
56	20.0	2.0	80.0	2.0	0	0.94
36	24.5	2.0	75.5	3.0	0	0.95
Average	15.5	6.1	84.5	1.5	0	
25	14.0	9.5	86.0	2.5	0	0.01
18 b	16.0	5.0	84.0	0.5	0	1.11
10	6.5	3.5	94.5	1.0	0	1.12
59	20.0	13.5	80.0	0.0	0	1.18
14	11.6	11.6	88.4	1.1	0	1.19
30	17.0	15.0	83.0	1.5	0	1.20
26	17.0	11.5	83.0	3.5	0	1.22
22	37.5	16.5	62.5	0.5	0	1.26
52	31.0	13.0	69.0	1.5	0	1.28
33	25.5	10.5	74.5	0.0	0	1.33
47	9.0	5.0	91.0	4.0	0	1.33
18 a	17.4	6.4	82.6	1.0	1 eos	1.37
1	5.0	4.0	95.0	2.0	0	1.39
57	22.5	16.5	77.5	3.0	0	1.40
28	20.0	16.0	80.0	0.5	0	1.42
32	19.4	19.4	80.6	0.0	0	1.42
58	34.5	23.5	65.5	2.5	0	1.43
6	9.2	8.2	90.8	0.5	0	1.44
51	23.0	15.0	77.0	3.0	0	1.48
Average	18.7	11.8	81.7	1.5	0.05	

tables show that these types of cell are rare when the anaemia is moderate, but they grow in number as it progresses. Under normal conditions, my investigations already referred to have failed to show any megaloblasts whatever and less than 1 per cent. pro-

Table 12. This table is a continuation of table 11.

Case No.	Promakro-megalo- blasts and megaloblasts in Sternal marrow per cent		Makroblasts, erythroblasts and erythroblasts in mitosis per cent		Normoblasts per cent	Erythrocytes in capillary blood in millions
53	24.5	17.0	75.5	1.5	0	1.50
2	1.0	0.5	99.0	0.25	0	1.53
27	16.0	10.0	84.0	3.0	0	1.58
23	27.0	8.5	73.0	3.5	0	1.67
48	17.4	10.5	82.6	0.6	0	1.75
7	22.0	10.5	78.0	0.0	0	1.82
24	11.5	5.0	88.5	3.0	0	1.88
49	21.0	9.5	79.0	3.5	0	1.89
8	16.0	7.5	82.0	1.0	2	1.95
34	21.0	14.0	79.0	1.6	0	1.95
19	21.0	12.1	78.4	0.0	0.5	1.96
Average	18.0	9.6	81.8	1.6	0.2	
5	13.0	13.0	83.5	2.6	3.5	2.00
55	17.5	13.5	82.5	0.5	0.0	2.01
13	40.9	9.2	59.1	1.2	0.0	2.04
16	5.0	5.0	78.0	5.0	17.0	2.06
12	15.5	12.0	84.5	0.0	0.0	2.17
35	19.8	16.3	72.6	2.5	7.6	2.27
17	4.0	3.5	28.0	3.0	68.0	2.37
15	2.6	2.6	53.4	0.9	44.0	2.38
31	6.0	0.0	49.0	1.0	45.0	2.38
29	20.0	16.0	80.0	0.5	0.0	2.46
Average	14.4	9.1	67.1	17.2	18.5	
37	11.9	5.7	71.9	6.3	16.2	2.69
21	7.0	3.0	68.0	0.0	25.0	2.87
Average	9.5	4.4	69.9	3.2	20.6	
20	9.0	5.0	78.0	2.0	13.0	3.15
9	0.0	0.0	24.0	0.0	76.0	3.61

macromegaloblasts (table 2). Table 12 shows that when the erythrocyte count is about three million, one begins to find megaloblasts, and at the same time the number of promacromegaloblasts also rises. Note in this connexion my interpretation of the promacromegaloblast as a precursor of all the erythroblast forms as explained in the section on the genesis of the erythroblasts. As the anaemia progresses, the number of promacromegaloblasts and megaloblasts

bution over the inner surface of all the left atrium. GROSS (1935) found in a material of 87 cases of rheumatic heart disease some sign of mural endocarditis in all cases. He regards however mural endocarditis to be present also in cases, in which the alterations consisted mainly of scarring and vascularization of the endocardium without conspicuous cellular infiltration.

According to these authors the inflammatory cells consisted mainly of lymphocytes but also, in the acute stages, of polymorphonuclear leucocytes, MACCALLUM found, in addition, typical ASCHOFF bodies. v. GLAHN found no typical ASCHOFF bodies but large, basophilic cells arranged to form "palisades" parallel to the endocardial surface. v. GLAHN and GROSS regarded however all the types of cells characteristic of the ASCHOFF bodies to be present in some cases of mural endocarditis, they assumed that only the anatomical structure of the endocardium prevents the development of distinct ASCHOFF bodies of the habitual type.

The authors mentioned did not observe the formation of mural thrombi as a consequence of mural endocarditis. The presence of a causal relation between mural endocarditis and mural thrombosis has hitherto been suggested only by GRAEF, BERGER, BUNIM & DE LA CHAPELLE (1937). They found in cases of rheumatic heart disease a mural endocarditis beneath 10 out of 24 left atrial thrombi, apparently both in the trabeculated and in the smoothwalled part of the atrium. They concluded that mural endocarditis was an important cause for the formation of left atrial thrombi.

In the present author's material the diagnosis of a significant mural endocarditis was based on the presence of a fairly uniform histological picture. The main feature was an infiltration of the endocardium with round cells (lymphocytes and plasma cells). Larger cells, comparable to the large cells occurring in ASCHOFF bodies were seldom observed. As a rule the cells were arranged in large, irregular foci *near to the myocardium*, sometimes there was a more or less continuous infiltration throughout the endocardium, which in 2 cases was filled with tightly packed round cells (fig. s. 11, 12 and 13). Sometimes the continuous cellular infiltration extended also to adjacent parts of the myocardium. Very often a degeneration of muscle fibres and an increase of the interstitial connective tissue could be observed in the layer of myocardium immediately outside the endocardium (fig. 12). As a consequence, the usually sharply

normoblasts in the sternal marrow. Here we see how the normoblasts, which normally dominate erythropoiesis completely, rapidly disappear altogether, being completely absent from the sternal marrow when the anaemia reaches two million erythrocytes per  $\text{mm}^3$ . This is such a constant state of affairs that there are normoblasts at a count of two million erythrocytes, it is safe to say that the marrow is not that of pernicious anaemia.

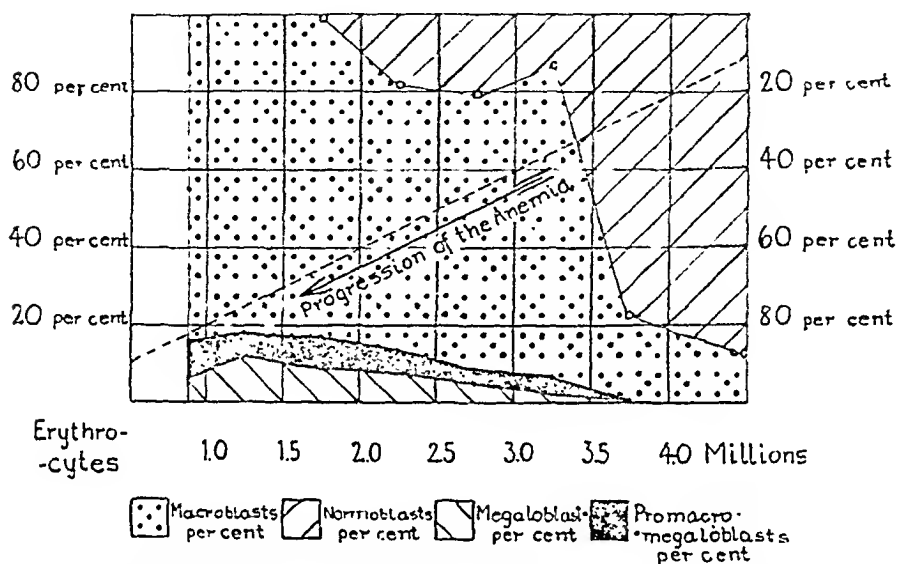


Fig. IV. Diagrammatic presentation of the development of erythropoiesis during the progress of pernicious anaemia through its three stages:

The normo-macroblastic stage.

The normo-macromegaloblastic stage.

The macromegaloblastic stage.

The diagram is based on the average figures in the tables 11 and 12.

The starting point is 4.5 million, own normal material (table 2).

Fig. IV, based on the averages in tables 11 and 12, presents the development of erythropoiesis during the progress of pernicious anaemia. We see how the sternal marrow undergoes the above-mentioned three stages.

Among the types of erythroblast mentioned, the pyknotic erythroblasts require special mention. Tables 7, 8, 9, 10 show how the percentage of the pyknotic erythroblast forms varies, occasionally rising to 40–50, the highest figures being reached when the erythroblast count is comparatively low. In case 6, for example,



Table 13. The average figures for the various leucopoietic types of cell found on sternal puncture in cases of untreated pernicious anaemia, the calculation being based on tables 7, 8, 9 and 10.

The average of table:		7.	8.	9.	10.			
Leucopoiesis	Anaemia in millions per mm <sup>3</sup> { from to	0.71 0.95	1.01 1.48	1.50 1.96	2.00 2.46	2.69 2.87	} 3.15 3.61	
	Megacaryocytes per cent	0.17	0.05	0.02	0.13	—	—	—
	Mast cells immature »	0.25	0.29	0.30	0.40	0.38	0.25	0.25
	mature »	0.04	0.04	0.05	0.03	—	0.50	—
	Eos. myelocytes »	1.50	1.81	1.30	1.45	1.88	1.75	0.75
	leuko- band forms »	1.58	1.75	1.93	1.80	1.50	1.50	1.75
	cytes polymorphs »	1.33	1.37	1.36	1.15	1.38	2.50	0.50
	Myeloblasts »	2.75	2.18	2.11	1.95	2.13	3.75	0.25
	Praemyelocytes »	6.50	4.69	5.43	3.95	2.38	3.75	4.—
	Neu- myelocytes »	15.71	16.46	14.50	13.70	9.50	15.25	22.25
	tro- young forms »	30.88	27.95	26.41	24.10	21.63	27.—	40.25
	philes band forms »	7.79	10.16	14.68	12.75	19.50	4.25	14.50
	polymorphs »	10.21	9.13	13.00	16.45	25.80	28.25	6.50
	Mono- blasts »	} 0.46	0.55	0.77	0.87	0.63	1.50	0.50
	cytes »							
	Lymphocytes »	7.13	9.37	8.23	10.50	3.75	2.75	0.75
	Plasma & Türk cells »	0.96	0.29	0.45	0.25	0.25	0.25	0.50
	Smears cells »	11.—	10.12	7.25	8.60	3.75	6.25	7.00
	Reticuloendothel. cells »	1.83	1.51	2.34	0.87	—	0.50	0.25

Table 13 gives the averages for the numbers of the various cell types of leucopoiesis on the same principle as before. The cell types of erythropoiesis are given in tables 11 and 12, and on the basis of tables 7, 8, 9, 10.

Here, if we compare these figures with the normal figures in table 2, we find they rise on a purely percentage basis of the reticulo-endothelial elements and of macerated cell types, from 0.2 to 1.8 per cent. for the reticulo-endothelial cells and from 3.6 to 11 per cent. for the macerated cells. This relative or percentage rise is so great that it probably indicates an absolute rise in the number of these cells when we compare these figures with those already referred to for the numbers of nucleated blood cells obtained by sternal puncture in untreated cases of pernicious anaemia. We may assume that the increase in the number of reticulo-endothelial cells depends on the pathological metabolism of the erythropoiesis. This profusion of phagocytic cells is mentioned by several authors

(DAMESHEK, ROHR, SCHULTEN). The rise in the number of mace-rated cells may depend on a rise in the number of atypical erythropoietic cells. I have already pointed out that the megalocytes are probably more vulnerable than the other erythropoietic cell types.

As the anaemia progresses, it would seem that the megacaryocytes remain unchanged at the normal figure, though some authors including TEMPKA and BRAUN, DAMESHEK, VALENTINE and HENNING find that their number is below normal and that they are over-segmented. With regard to the basophile and the eosinophile granulocyte forms I have also failed to find in my material quantitative changes demonstrable by differential counts after sternal puncture.

The neutrophile granulocytes seem to show quite insignificant differences in their percentage distribution. Thus the figures for fully developed anaemia (see first column in table 13) are practically identical with the normal figures in table 2. But we find a growing profusion of pathological leucocytic cell types in the form of macroleucocytes with their large, metamyelocyte-like nuclei and over-segmentation of the neutrophile leucocytes. These changes run parallel with the progressive peripheral granulocytopenia.

Lastly a word about the lymphocytes whose percentage distribution diminishes somewhat as the anaemia increases, from the normal 16 per cent. to fully 7 per cent. when the anaemia is advanced. Most probably this fall is to some extent only relative because the myeloid cells occupy more space and displace the lymphoid tissue. But the lymphoid tissue in the bone marrow is assuredly not reduced in absolute figures, for we must remember that there is an increase in volume of the red bone marrow.

When we now take a comprehensive view of the morphological and purely quantitative findings from sternal puncture in untreated cases of pernicious anaemia, it seems to me that we cannot accept the opinion held by most authors (VEENEKLAAS, BANG, ROHR and many others) that the marrow of pernicious anaemia is hyperactive and hyperplastic.

As pointed out in the preceding chapter, and in relation to normal average figures, the marrow of pernicious anaemia is comparatively poor in cells, with, perhaps, an insignificant rise in the number of nucleated blood cells per unit of space when sternal

puncture is made in the final stages of the disease. This rise is not, however, so marked that one can speak of a genuine hyperplasia, and it cannot be compared with the gigantic rise in the number of cells observed in genuine, regenerative erythropoieses. On the other hand, both the erythropoietic and the leucopoietic cells are very large, and I believe that it is this increase in cell volume which accounts for the red bone marrow and displaces the fatty tissue. We know that the bone marrow is extensive and that its volume is increased, witness the post-mortem findings of red bone marrow in bones where it does not occur normally. This is, however, interpreted as *hypertrophy*.

In my opinion the red bone marrow in pernicious anaemia develops in the following way: when the supply of the antipernicious anaemia factor fails, *the first change in the normal development of the cells takes place in the marrow's haematopoietic tissues which under normal conditions are extensive*. This tissue is inactivated and degenerates in a macromegaloblast way, the pathological-anatomical result being the large bone marrow cells, and the functional result being a reduction of the cells of both the leucopoietic and erythropoietic types. The result is primary marrow hypertrophy in this sphere.

The bone marrow, however, changes gradually in the following way: When there is a failure of cell production in the normal area of distribution of the haematopoietic tissues, haematopotent tissues elsewhere in the body (the bone marrow) and hitherto at rest begin to be active, the fat in the marrow being gradually displaced. This new process of cell division and growth (which can be described in its primary phase as hyperplasia) becomes exposed, when the cells have developed a little, to the influence of the same shortage of the antipernicious anaemia factor. To begin with the tissues exploit their latent capacity for division and growth, displacing other structures; and the youngest erythropoietic cells, the promacromegaloblasts, and the young, pathological leucopoietic types of cell develop, with consequent *primary marrow hyperplasia*. In these areas also the bone marrow now becomes red. But here also the tissues soon enter on the inactive phase which is a secondary phenomenon in the course of the disease in contrast to the primary inactive phase in the normal area of distribution of haemato-



poietic tissue. Thus in areas of bone marrow where, normally, blood cells are not formed, there is in pernicious anaemia a gradual development of a *secondary and inactive hypertrophy of the marrow*. Here the large young types of cell, which do not develop further to any great extent, and which undergo degeneration and hypertrophy, are piled up. In my opinion, if we regard the bone marrow including red bone marrow as a functional entity, we cannot regard it as anything but *hypertrophic and inactive*. To be sure, this point of view clashes with the conception in general pathology of hypertrophy being associated with increased, not decreased function.

#### *Conclusion:*

1) *In the development of pernicious anaemia, it is possible morphologically to recognize three different stages in the sternal marrow, — the early normo-macroblastic stage, the intermediate normo-macromegaloblastic stage, and the final macro-megaloblastic stage.*

2) *Normoblasts are not to be found in fully developed pernicious anaemia.*

3) *The discovery of normoblasts when anaemia is less than some two million erythrocytes is a direct challenge to the diagnosis of pernicious anaemia.*

4) *In pernicious anaemia, sternal puncture yields more (comparatively) macroblasts than in any other form of anaemia. The youngest forms of this type of cell, and notably the macromegaloblastic transitional forms, dominate the picture of sternal puncture in fully developed pernicious anaemia.*

5) *The megaloblasts begin to develop in the sternal marrow when the anaemia reaches 3.5 million erythrocytes. These megaloblasts are found only when the supply of the antipernicious anaemia factor fails.*

6) *The mitosis count remains quite unchanged as the anaemia progresses.*

7) *As the anaemia progresses, there is a doubtful rise in the absolute number of erythroblasts, but a definite rise of their relative number per volume unit on sternal puncture.*

8) *An increased number of pyknotic erythroblasts is suggestive of a complication, often of a toxic character.*

9) *The marrow of pernicious anaemia is hypertrophic and it*

functions subnormally, being neither hyperplastic nor functionally hyperactive.

10) The percentage interrelationship of the granulocytes of leucopoiesis is normal, but their absolute number per unit of volume on sternal puncture falls as the anaemia progresses. Running parallel with progressive peripheral leucopenia there is an increasing number of pathological leucocyte forms.

11) The number of megacaryocytes remains, unchanged as the anaemia progresses.

12) As the anaemia increases there is a growing number of phagocytes of R. E. types.

13) Running parallel with the progress of the anaemia is a rise in the number of macerated cells which are probably remains of erythropoietic megalocytes.

14) As the anaemia progresses there is a relative decline in the number of lymphocytes.

### C. The Reticulocytes.

1) *Previous investigations.* Under normal conditions the peripheral blood is supposed to contain from 0 to 20 per thousand reticulocytes (PRICE-JONES, VAUGHAN and GODDARD). This estimate recurs repeatedly in the literature. TRACHTENBERG, for example, give 3 to 14 per thousand, SEYFARTH 1 to 2, ROSSINGH 4 to 18.

In normal bone marrow TEMPKA and BRAUN found 8 per thousand, POKROWSKY under normal conditions found the reticulocyte ratio in bone marrow/blood as 8—14/2—5 per thousand, and ROHR 15—20/10—15 per thousand, UNGRICH 14—40/6—12 per thousand, and KLIMA 3—20 per thousand in normal sternal marrow.

Thus we see that in both peripheral blood and sternal marrow the ratio of reticulocytes is under the normal 20 per thousand with the exception of UNGRICH whose uppermost limit is 40 per thousand. All observers agree in finding the reticulocyte count for sternal marrow is a little higher per thousand than for peripheral blood. As all the other observers quoted have found figures under the low upper limit for normal peripheral blood, UNGRICH's

higher figures are of questionable value as a criterion for findings in pernicious anaemia. It is highly probable that his counts have included reticulocytes with fragments of nuclei.

We must expect to find reticulocyte counts to be dependent on so many different factors that it is difficult to gauge their significance and to draw any conclusions from them. One cannot therefore attach any importance to them. To do so one would have to undertake parallel counts over a long period of the reticulocytes of the bone marrow and peripheral blood respectively, and also take into consideration the oxygen tension in the blood and tissues (see MINOT's and CASTLE's study in 1935). Small haemorrhages, slight poisonings, hormones and vitamins (SEYDERHELM and GREBE) and several other factors may intervene.

In somewhat advanced, untreated pernicious anaemia showing no sign of remission, there may be up to 40—50 per thousand reticulocytes in the peripheral blood. Under agonal conditions the figures may be somewhat lower, even down to 0. These reticulocyte counts must be regarded as very low when we bear in mind the patient's anaemia and the comparatively high erythroblast count in the bone marrow. This state of affairs is probably due to a much reduced productive capacity on the part of the bone marrow.

The literature contains very few reports of exact reticulocyte counts from the sternal marrow in pernicious anaemia. Pokrowsky maintains that, in pernicious anaemia, the reticulocytes are somewhat more numerous in the marrow than in the peripheral blood, but like PAOLAZZI and SPADACCINI, he gives no figures.

*Own investigations.* In my own normal material (table 28) I found the sternal marrow of 15 patients to contain on the average 9 per thousand, the extreme limits being 2 and 22 per thousand. As these findings are in complete agreement with those of other observers, I have not found it necessary to pursue this particular investigation further.

Table 14 gives 62 reticulocyte counts from the bone marrow and peripheral blood of 39 of my patients, the arrangement of this table depending on the degree of the anaemia, the most severe anaemia coming first. In this table there are different groups as in table 4.

It will be noted that the number of reticulocytes seems to be

*Table 14.* Reticulocytes per thousand in sternal puncture smear. Untreated cases of pernicious anaemia. Arrangement according to the degree of the anaemia, as in table 4. There are three divisions in this table, separated from each other by broken lines. The average figures for the resultant groups are calculated and included in table 16. Own material.

Case No.	Reticulocytes per mille		Case No.	Reticulocytes per mille	
	Cap. blood	Sternal blood		Cap. blood	Sternal blood
11	21	48	24	20	31
4	71	90		23	26
	72	114		21	27
	70	88	18	9	9
50	20	48	34	6	11
	28	43	19	18	29
	17	34	5	9	10
36	21	24		9	17
25	6	18	13	4	18
18	49	60	16	29	38
	10	20		28	34
10	32	41	12	5	6
14	9	17		5	7
30	40	—		6	8
26	11	9	35	5	8
	10	12	17	41	39
	11	14		34	36
22	16	23		39	40
52	45	31		38	42
33	8	14	15	7	8
47	13	—		7	12
	25	—		2	3
1	10	7	31	9	13
28	12	15	29	15	—
	12	21	37	6	31
32	2	2	21	31	22
6	8	15	20	15	18
51	21	25	9	14	9
	25	19		3	5
2	9	34			
23	7	13			
17	6	6			
	5	10			

somewhat higher in well-developed than in moderate anaemia, but the difference is not very marked. Further, the number of reticulocytes seems to be a little higher in the sternal marrow than in the peripheral blood. But here, too, the difference is not very marked, and by no means the rule in any way with regard to parallel relationship between the number of the reticulocytes and the degree of the anaemia. Table 15 gives the averages for all the punctures:

*Table 15 gives the averages and ranges of variation of the reticulocytes in the peripheral blood and sternal marrow of 39 patients in various stages of untreated pernicious anaemia.*

	Average	Range of variation
Reticulocytes per thousand in blood of ear ..	19.1	2—72
Reticulocytes per thousand in sternal blood..	25.4	2—114

The figures in this table may possibly be taken to show that sternal marrow contains a few more reticulocytes per thousand than peripheral blood, but they are not convincing.

When we break table 14 up into three parts and calculate the averages for each, we get table 16:

*Table 16. The average number and range of variation of the reticulocytes counted at 20 examinations in each of the first two groups and at 22 examinations in the third group.*

Group	Average	Range of variation
I Reticulocytes per thousand in blood of ear ..	28.4	6—72
Reticulocytes per thousand in sternal blood ..	37.4	9—114
II Reticulocytes per thousand in blood of ear ..	13.0	2—25
Reticulocytes per thousand in sternal blood ..	17.3	2—34
III Reticulocytes per thousand in blood of ear ..	16.0	2—41
Reticulocytes per thousand in sternal blood ..	19.7	3—42

This table shows that we can count on a slight increase in the number of the reticulocytes in the sternal marrow as compared with their number in the peripheral blood. It would also seem that

the number of the reticulocytes rises in a somewhat irregular fashion as the anaemia increases. This applies to both the sternal marrow and the peripheral blood.

*Conclusion: In both normal blood and sternal marrow the reticulocytes are under the normal upper limit of 20 per thousand. The figures are perhaps a little higher for sternal marrow than for peripheral blood.*

*In pernicious anaemia the reticulocyte figures are a little above normal and a little higher in sternal marrow than in peripheral blood. This rise in their number is small in relation to the degree of the anaemia and the relatively high number of erythroblasts found. This is taken to mean that the productive capacity of the bone marrow in pernicious anaemia is very small.*

## VII. The Sternal Marrow in Liver-treated Pernicious Anaemia.

*Previous investigations.* Exact investigations of the changes in the number of the nucleated blood cells in the sternal marrow during treatment with liver extract are not to be found in the literature. One of the reasons for this, as already pointed out, is that in the opinion of most observers, investigations in this direction give a poor and unstable picture of the sternal marrow. But, as already noted, I have succeeded in showing that, within certain limits, the quantitative findings of sternal puncture give us a very reliable picture of conditions in the sternal marrow.

I have also failed to find in the literature any investigations concerning the strictly quantitative oscillations between the cells of erythropoiesis and leucopoiesis, and I fancy that this is so for the reason I have just given. On the other hand, there is a quite overwhelming literature concerning the purely qualitative changes and the behaviour of the reticulocytes. Everyone agrees that, sooner or later, the bone marrow becomes «normal». But it is more difficult to understand how and why this happens, and here we again encounter the same difficulties, frequently referred to, concerning variations in nomenclature.

TEMPKA and BRAUN found (1932) that the «promegaloblasts» disappear in cases of pernicious anaemia in the course of four to six weeks' treatment with liver. The cells they call «promegaloblasts» are probably closely allied to the promacromegaloblasts in the present study. ZADEK was the first to study systematically the marrow in pernicious anaemia in connexion with liver treatment (1922), and he found that the red bone marrow in the long bones turned into fatty marrow again during a remission of this disease. He always found that «megakoblasts» were demonstrable long after the completion of a remission. Obviously this is not the case

if we keep to a sensible nomenclature, and the cell he has found and discussed in treated cases of pernicious anaemia is assuredly identical with the cell called promacromegaloblast in the present study.

Everyone is aware of the marked erythropoietic proliferation which follows liver treatment. MARKOFF observed it 24 hours after such treatment. He evidently refers to the increase in the number of macroblasts on sternal puncture described in the present study. LEITNER observed the same phenomenon after 12 to 24 hours, and ELSA SEGERDAHL after 24 hours in two cases in one of which there was a rise from 24 to 74 per cent. of the comparative »normoblast values». During remissions in 10 cases, ELSA SEGERDAHL found the cell count from sternal puncture rise to an average of 490,000, the lowest figure being 26,000 per  $\text{mm}^3$ . On the completion of a remission the figures again approached the normal, with a cell count of 41,000 to 288,000 per  $\text{mm}^3$ .

### A. Quantitative Conditions.

*Own investigations.* To give a comprehensive view, the results of my observations are classed in four groups.

*Group 1.* Cases in which an injection of liver extract raised the number of erythrocytes in the blood in the ear up to  $\frac{1}{2}$  million per  $\text{mm}^3$ .

*Group 2.* Cases in which an injection of liver extract raised the number of erythrocytes in the blood in the ear from  $\frac{1}{2}$  to 1 million per  $\text{mm}^3$ .

*Group 3.* Cases in which an injection of liver extract raised the number of erythrocytes in the blood in the ear from 1 to  $1\frac{1}{2}$  million per  $\text{mm}^3$ .

*Group 4.* Cases in which an injection of liver extract raised the number of erythrocytes in the blood in the ear to more than  $1\frac{1}{2}$  million per  $\text{mm}^3$ .

The first of these groups is divided into sub-groups according to the degree of the anaemia, i.e. the level of the erythrocyte count in the peripheral blood when liver treatment was instituted.

Sub-group A. Cases with anaemia of 0 to 1.6 million per  $\text{mm}^3$ .

»	»	B.	»	»	»	»	1.6	»	2.6	»	»	»
»	»	C.	»	»	»	»	2.6	»	3.6	»	»	»



As the case records show, the number of nucleated blood cells yielded by sternal puncture rose invariably, rapidly and markedly during the days after the injection of a liver preparation. They fell in a few days again to normal.

In the sub-group A of group 1 (Table 17) we see that the number of nucleated blood cells yielded on sternal puncture is at the start on the average 73,800 per  $\text{mm}^3$ . The average for the highest figures which were invariably registered on the third day after a liver injection was 223,400 pr  $\text{mm}^3$ . Thus at the start the figure corresponded to the normal average, whereas the highest figures registered during the treatment exceeded the upper limit of the range of variation under normal conditions. This high cell count presumably indicates a genuine increase in the number of cells yielded by sternal puncture, and in this regenerative phase of reaction the sternal marrow must be assumed to be *hyperplastic* and *hyperactive*. This is in contrast to the inactive hypertrophic bone marrow of untreated pernicious anaemia. Now, in the *hyperplastic phase of reaction*, there is a marked reduction in the size of the cells, the erythropoiesis of the marrow being, as will be discussed later, made up of the smaller macroblasts and normoblasts. As table 17 shows, the number of cells yielded by sternal puncture rises on the average by 135,600 nucleated cells per  $\text{mm}^3$ . This figure alone is far above the normal average for nucleated blood cells yielded by sternal puncture in health and in untreated cases of pernicious anaemia.

Though we cannot, as already pointed out, with the technique employed count on obtaining absolutely reliable figures concerning the number of nucleated blood cells yielded by sternal puncture, I believe that the great and constant reaction recorded during the first days of liver treatment indicative of a real increase in the number of cells obtained by sternal puncture and therefore also in the bone marrow.

The same conditions are to be found in sub-groups B and C of table 17. The highest figures were registered about the third day, and the average level was practically invariable. The same was the case with the rise in the number of nucleated blood cells. If we fuse sub-groups A, B and C into one, we find an average at the start of 69,800 cells per  $\text{mm}^3$ . for untreated pernicious anaemia, and an

*Table 17.* The number of nucleated blood cells found on sternal puncture before liver treatment, and the highest count in response to it. The difference between the two, i.e. the rise in the number of nucleated blood cells, is given. In the fourth column is the day on which this happened. This table includes those patients who responded to liver treatment with a rise of up to half a million erythrocytes in the peripheral blood. The sub-groups A, B, C include the patients in this table classified according to the degree of their anaemia before they received treatment. Own material. The number under «remarks» shows the days after liver injection where no sternal puncture was made.

Case	Count at starting point	Peak	Difference	Day after the injection	Remarks
10	71,000	111,900	40,900	3	2
22	78,600	236,200	157,600	2	3
23	72,900	544,300	471,400	3	2—5
25	101,500	174,700	73,200	5	Erythrobl.-peak 3rd day
11	43,000	39,500	—	{2—}4	Mixture of blood
14	74,800	156,000	81,200	3	
18	64,300	83,500	19,200	3	
51	51,300	157,000	105,700	4	5
Average	73,800	223,400	135,600	3.3	
<i>Group 1, A.</i>					
17	82,700	147,400	64,700	2	High retic. count at time of the inject.
13	123,700	246,000	111,300	1—4	
19	191,900	224,000	—		
		125,400	—	2	
15	44,900	82,100	37,200	2	
12	21,800	304,600	282,800	4	2—4
24	79,800	152,800	73,000	3	2—4
Average	75,800	192,700	113,800	2.7	
<i>Group 1, B.</i>					
26	67,800	113,200	45,400	3	2—4
21	42,900	310,500	267,100	3	2—4
Average	55,400	211,900	156,300	3	
<i>Group 1, C.</i>					
	69,800	209,600	130,000		
<i>Average Groups 1 A, B, C.</i>					

*Table 18.* This table shows the number of nucleated blood cells found on sternal puncture before liver treatment as well as the peaks reached in response to it. The difference between the two, i.e. the rise in the number of nucleated blood cells, is given. In the fourth column is the day on which this peak was reached. In groups 2, 3, and 4 are the patients who responded to liver treatment with a rise in the erythrocyte count in the peripheral blood of  $\frac{1}{2}$  to 1, of 1 to  $1\frac{1}{2}$ , and of more than  $1\frac{1}{2}$  million erythrocytes respectively. The averages for these groups are calculated. Own material. The number under *remarks* shows the days after liver injection where no sternal puncture was made.

Case	Count at starting point	Peak	Difference	Day after the injection	Remarks
28	78,400	341,500	263,100	3	
29	240,000	160,000	160,000	2	
53	118,500	412,000	293,500	3	2—4
55	76,000	900,000	824,000	5	2—3—4
56	137,800	238,800	101,000	4	3—5
57	93,200	253,500	140,300	3	2—4
Average	124,000	384,300	297,000	3.3	
<i>Group 2.</i>					
32	98,000	208,000	110,000	3	
33	208,000	367,000	159,000	3	
31	9,100	129,000			Mixture of blood
Average	153,000	252,000	134,300	3	
<i>Group 3.</i>					
38	101,800	157,400	55,600	4	3—5
36	64,200	175,400	111,200	3	2—4
39	77,600	177,400	99,800	3	4
51	71,400	157,000	85,600	4	
35	42,100	31,000	—		2—4
37	104,800	63,000	—		1—3—4
59	35,900	441,500	385,600	2	
Average	70,200	201,700	147,600	3.2	
<i>Group 4.</i>					

average maximum of 209,600 per  $\text{mm}^3$ . on the third day of treatment. The average rise was 130,000 cells per  $\text{mm}^3$ .

The same state of affairs exists in group 2 which includes six patients (table 18). In the same table are groups 3 and 4 with two and five patients respectively. Cases 31, 35 and 37 are included here, but they cannot be relied on as the sternal punctures were unsatisfactory. In these groups also, we find exactly the same conditions.

The figures vary a trifle, but not to such an extent that the separation of the cases in different groups has revealed any variations of importance. We may therefore conclude that injections of liver preparations induce the same rise in the number of nucleated blood cells on sternal puncture whatever the degree of the anaemia. But this is so only, as we shall see later, if sternal puncture yields a low normoblast count. A scrutiny of the case records suggests that this state of affairs is but little affected by the quantity of the liver preparation injected, the maximum figures being recorded about the third day whatever the dosage. At any rate, whatever differences there may be they are not so great as to be definitely noticeable with the technique employed. In case 15, the maximum figure was not reached till the fifth day, but this patient suffered from polyarthritis which was exceptionally active at the time of the injection. Later on also, the reaction of the bone marrow to the treatment in this case was sluggish, and exceptionally large doses of liver preparation were required in order to achieve normal blood counts. Here it should be noted that, in certain cases, infections seem to require an increased dosage of a liver preparation and, possibly, to delay the reaction of the bone marrow in pernicious anaemia. In the above-mentioned case attention should be drawn to the fact that, at the time of the injection, the sternal marrow was partially normoblastic, and for this reason could not be expected to react fully to the treatment; the basal conditions necessary for a complete bone marrow reaction did not exist. The already present normoblastic tissue did not need to be changed in order to produce normal erythrocytes.

Table 19 gives the averages and ranges of variation for all the treated groups together.

	Count at outset	Peak	Difference	Day after injection
Average count.....	88,100	251,300	170,800	3rd
Highest count.....	240,000	900,000	824,000	"
Lowest count.....	21,800	82,100	19,200	"

This table also gives a comprehensive survey without contributing anything new to what has already been said in this chapter.

*Table 20.* This table gives the percentage of erythroblasts among the nucleated blood cells found on sternal puncture before liver treatment, and the highest percentage reached. The day on which this happened is noted. This table deals with the patients who responded to the injection of a liver preparation with a rise of up to half a million erythrocytes in the peripheral blood. The patients in sub-groups A, B and C are classified according to the degree of their anaemia when the liver treatment was started.

Case	Erythrobl. per cent		Day	Maximum rise	Remarks
	on inject.	Maximum			
10	47.6	54.2	1—3	7.5	3rd day few cells on sternal puncture
22	35.1	45.6	2	10.5	
23	32.9	65.1	3	32.2	
25	37.5	53.2	3	15.7	
11	43.6	68.8	3—4	25.2	
14	38	66.4	3	28.4	
18	40.4	52	3	11.6	
Average	39.3	57.9	3	18.7	
<i>Group 1 A.</i>					
17	40.2	50.9	1—2	12.4	No sternal puncture on 3rd day
13	34.6	48.2	2—4	14.6	
19	35.—	43.6	2—4	13.2	Makro-normobl. marrow before treatment
15	24.—	51.7	5	27.7	
12	26.8	48.5	2—4	27.2	
24	34.2	52.9	3	18.7	
Average	32.5	49.3	3	18.9	
<i>Group 1 B.</i>					
26	29.2	53.1	3	24.3	No stern. punct. on second and fourth day
21	23.9	52.8	3	28.9	No stern. punct. on second and fourth day
<i>Group 1 C.</i>					

As the case records show, the percentage distribution of erythroblasts yielded on sternal puncture swings in the same way as that of the nucleated cells (see tables 20 and 21).

In these tables the erythroblast counts are recorded on the same principles as those followed in the tables showing the counts of nucleated blood cells in bone marrow in response to the treatment of pernicious anaemia with liver preparations. The tables show an

*Table 21.* This table gives the percentage of erythroblasts among the nucleated blood cells found on sternal puncture before liver treatment, and the highest percentage found in response to the injection of a liver preparation. It also gives the highest rise observed and the day on which it occurred. The patients are allotted to groups 2, 3 and 4 according as the liver treatment increased the erythrocytes in the peripheral blood by  $\frac{1}{2}$  to 1 million, by 1 to  $1\frac{1}{2}$  and by more than  $1\frac{1}{2}$  million respectively per mm.<sup>3</sup> The averages for these three groups are given. The number under 'remarks' shows the day after liver injection where no sternal puncture was made.

Case	Erythrobl. per cent.		Day	Maximum rise	Remarks
	on inject.	Maximum			
28	40.1	76.9	3	36.8	2—3—5
27	22.—	63.—	4	41.—	
29	61.3	73.1	2	11.8	
30	30.2	52.1	3	21.9	
53	44.9	57.5	3—5	12.6	2—4
54	33.6	63.7	5	30.2	3—1—6
55	33.3	66.6	5	33.3	2—3—4
56	54.8	78.6	4	23.8	
57	41.7	48.8	3	7.1	2—4
58	48.7	65.8	2	17.1	1—3
Average	41.1	64.3	3.5	23.6	
<i>Group 2.</i>					
32	35.—	76.5	2—3	43.—	2—4
33	50.9	69.—	3—4	19.5	
31	29.—	66.2	3	37.2	
Average	38.3	70.5	3	33.2	
<i>Group 3.</i>					
38	44.1	69.4	4	25.3	2—3—5
36	48.5	71.4	3	29.9	2—1
34	57.8	79.—	2—4	21.5	4
35	23.6	55.6	3	32.—	2
37	32.—	42.7	2	10.7	2
51	41.4	72.4	3	31.—	
59	36.3	74.1	3	37.8	
Average	40.5	66.4	3	26.9	
<i>Group 4.</i>					

absolute and constant rise in the percentage of erythroblasts under liver treatment. This state of affairs seems to be most marked in the groups in which the greatest rise in the erythrocyte count in the peripheral blood after treatment with injections of liver prepara-

tions was recorded. See table 20 in which liver treatment led to a rise in the erythrocyte count of up to  $\frac{1}{2}$  million per  $\text{mm}^3$  in the peripheral blood. Here the rise is about 20 per cent., whereas it is still higher in table 21 in which are collected the patients showing a rise of more than  $\frac{1}{2}$  million erythrocytes in response to liver treatment. The percentage of erythroblasts also reached the maximum about the third day. This rise is absolute as well as relative for, as pointed out earlier in this chapter, this rise coincides with the rise in the number of nucleated blood cells. Here, too, the maximum was reached about the third day.

This relative rise in the number of the erythroblasts is not enough to account for the whole of the rise in the number of nucleated blood cells of the sternal marrow. From the recorded figures for the rise in the number of nucleated blood cells and the relative percentages of erythropoiesis and leucopoiesis after liver treatment, we may count on a rise (somewhat more moderate) in the number of cells of the leucocyte type yielded by sternal puncture. The amount of the liver preparation injected does not seem to affect the reaction of the sternal marrow with regard to the interrelationship of the cell forms of erythropoiesis and leucopoiesis, nor to the degree of the anaemia at the time of an injection.

The fact that the *quantity* of the injected liver preparation is of relatively small importance to the primary bone marrow reaction, whereas the ensuing rise in the number of erythrocytes may yet vary in the peripheral blood, shows that, like other diseases caused by »hormone failure», pernicious anaemia may vary in severity and malignancy, even though this cannot at present be demonstrated in other ways. Of course the dosage may be so timid that it has no visible effect. Nearly all the patients in this study were intentionally treated with small doses of liver preparations, some quite small, in order to investigate the various liver fractions as mentioned in the chapter on technique. Case nr. 14, for example, received 4 cc. »BBaBF. u.s.E» which corresponds to 0.7 mgm. in the dried state. Yet this dosage effected a complete revolution in the sternal marrow. Liver preparations are not »standardized» in relation to each other, so there is no absolutely reliable basis for comparisons. Such standardization can hardly be effected with complete accuracy at the present time. We may assume that large

quantities of liver preparations can, to a certain extent, be stored in the body in depots, and that their action on bone marrow may be prolonged, i.e. that the normal normoblastic erythropoiesis may be maintained. But the immediate or primary bone marrow reaction is, as already pointed out, the same whether large or quite minute doses of liver are given. Experiences with small doses have, as already noted, given rise to the theory that there are many forms of pernicious anaemia which reflect variations in the failure of the antipernicious anaemia factor. For the rise in the erythrocyte count varied greatly although most of the patients were given small doses of liver on commencing treatment at the time when successive sternal punctures were undertaken.

Probably the sternal puncture changes noted in this chapter are somewhat toned down in incipient pernicious anaemia which very seldom receives treatment as the patient does not feel ill enough to seek a doctor. A case in point (nr. 9) is that of a woman, aged 61, who sought medical aid for symptoms which were traced to funicular myelosis associated with pernicious anaemia. Her anaemia was moderate, and sternal puncture showed a mainly normoblastic erythropoiesis. She was given adequate treatment, and there was a satisfactory rise of the peripheral blood count. The erythropoiesis being mainly normoblastic, no great changes could be expected of injections of liver. This proved to be the case. The nucleated blood cells yielded on sternal puncture and the percentage of erythroblasts failed to rise. There was no demonstrable reticulocyte crisis, but the erythropoiesis became completely normoblastic.

#### *Conclusion:*

1) *In response to liver treatment, there is on sternal puncture a constant and marked rise in the number of nucleated blood cells.*

2) *This rise reaches its maximum about the third day after this treatment.*

3) *At this stage the bone marrow is hyperplastic and functionally over-active.*

4) *The nucleated blood cells yielded by sternal puncture show a constant, relative and absolute rise of the erythroblast percentage.*

5) *Here, too, the maximum is reached about the third day.*

6) *The above-mentioned rise depends but little on the degree of the*



anaemia as long as the yield of sternal puncture does not include a great number of normoblasts.

7) The primary bone marrow reaction depends but little on the quantity of the liver preparation injected.

8) When the erythrocyte rise in the peripheral blood is highest, the reaction of the sternal marrow may possibly be somewhat more marked than is usual.

9) In response to liver treatment, sternal puncture also shows an increased number of cells of the leucocyte type.

10) There may well be various precursor types of pernicious anaemia reflecting the degree of failure of production of the antipernicious anaemia factor.

## B. Qualitative Conditions.

*Own investigations.* As the case records show quite plainly, the findings of sternal puncture conform in a perfectly regular way to liver treatment before which, as already pointed out, the sternal marrow is megalomacroblastic. Within 24 hours of the administration of liver extract, the sternal marrow is always young-macroblastic, young macroblasts dominating the picture. These macroblasts mature rapidly, and at the same time there is a rise in the number of nucleated blood cells on sternal puncture, as pointed out in the preceding chapter. About a day after the macroblast peak there is a *basophile normoblast peak*, and a little later (in about 24 hours) the *eosinophile normoblast peak* is reached. Coinciding with the domination of the normoblasts we find the nucleated blood cells reaching their peak, also about the third day. The eosinophile normoblast crisis in the sternal marrow coincides with the peak of the nucleated blood cells in the peripheral blood, and they naturally enough consist of eosinophile normoblasts.

I take it for granted that sternal puncture gives an unequivocal picture of the sternal marrow even if the percentage distribution of the different types of cell may possibly vary, and if so probably in the direction of a greater number of the younger cells in the sternal marrow. There is a possibility that the older types of cell are most easily detached in the process of aspiration of the sternal marrow, — a possibility which may, perhaps, account for varia-

tions in counts. But such possible and slight variations cannot conceivably affect the conclusions to be drawn from the findings of sternal puncture with regard to the functions of the sternal marrow. For we may be sure that the findings of sternal puncture run strictly parallel with events in the sternal marrow.

The above-mentioned changes in the findings of sternal puncture in response to liver treatment constitute the normal-physiological basis for the provocation of the reticulocyte crisis in the bone marrow. The peak of this crisis always appears directly after the eosinophile normoblast peak, usually in the course of 24 hours. As mentioned in the preceding chapter, this reaction may *possibly* be a little sluggish, but not to any marked degree, in elderly folk and in the presence of complications (see a more comprehensive discussion of this point in a later chapter). The reaction may certainly be more sluggish when liver treatment is started in cases of moderate anaemia in which the marrow is rich in normoblasts (see case nr. 44).

The above-mentioned changes and happenings must undoubtedly be interpreted as a physiological development of the erythrocytes on a gigantic scale, except for the initial transformation of the degenerating megalocytes which does not, of course, belong to the normal process of development.

Fig. VII (a—b—c—d) is a graphic presentation of the changes mentioned with regard to cases 31, 32, 33 and 34. It will be seen that the thin, unbroken line, representing the percentage of erythroblasts in relation to the nucleated blood cells obtained by sternal puncture, begins to rise rapidly in response to liver treatment, reaching its peak on the third day. Coinciding with, and towards the end of this peak, there is a flooding of the peripheral blood with erythroblasts as indicated by the thick broken line which reaches its peak on the third day. This line represents normoblast types (see also case records). Next day there is a reticulocyte peak (see the thin, broken line and the finely dotted line representing the reticulocyte peaks for sternal marrow).

What theoretical conclusions can be drawn from these consistently regular changes in the bone marrow of pernicious anaemia?

In fig. III the cell types found in untreated cases of pernicious anaemia are included in the triangle formed of broken lines. As the

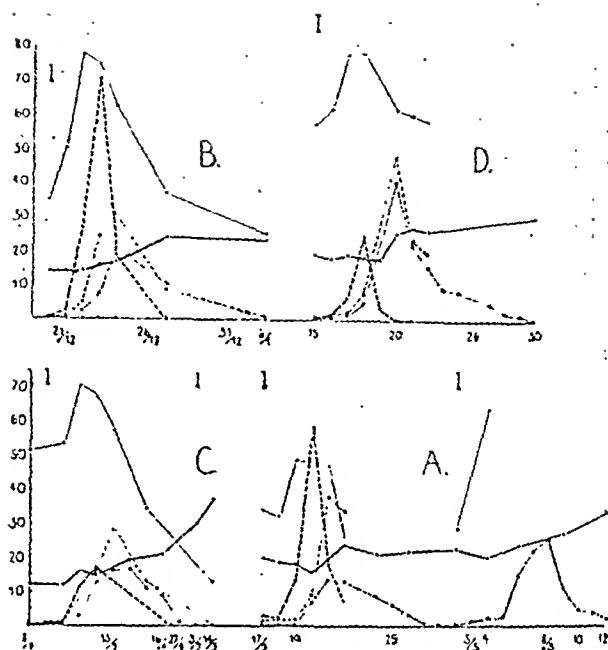


Fig. VII; A: case 13, B: case 32, C: case 33, D: case 34.

- : Percentage of erythroblasts on differential count of sternal puncture smear.  
 —————: Erythrocytes in blood of ear per m.m. in 100,000.  
 - - - - -: Percentage of reticulocytes in sternal blood.  
 .....: Percentage of reticulocytes in blood of ear.  
 - - - - -: Erythroblasts in blood of ear per m.m.<sup>3</sup>.  $\frac{1}{100}$   
 ↓ : Injection of liver preparation.

anaemia progresses, these cells gradually approach the megalocytes more and more (see also micro-photo, fig. V A. page 83 and fig. VI A and B, page 85 and 86). This state of affairs finds expression in the differential counts of sternal puncture preparations in untreated cases of pernicious anaemia, and it is dealt with in chapter VI B. One finds numerous erythroblasts which consist of macroblasts, megaloblasts and promacro-megaloblasts and transitory forms between these. In fully developed pernicious anaemia, erythropoiesis consists entirely of these cell types and a variable number of cells counted as erythroblasts, i.e. pyknotic forms whose nuclear structure defies classification with the above-mentioned cell types from which they undoubtedly hail. Of less importance is the absolute number of the various cell types of erythropoiesis. The characteristics of these cells merge so much into each other that in many

cases it is very difficult and a matter of personal opinion in deciding to which group they belong.

The most typical »outer or extreme groups» numerically provide the most reliable index to the extent to which the changes in the bone marrow have proceeded. In smears we find all the transitional forms between the quite diagrammatically classified types of erythroblast, and there is absolutely nothing to indicate the existence of two distinct series of development of the erythroblasts. From the purely physiological point of view this conception appeals to one most, for it seems quite plain that it is the young normal types of erythroblast which degenerate. They do not develop or ripen from the juvenile types.

Let us now examine the first stage of the process started when the pernicious anaemia factor begins to act. If we compare the sternal marrow differential counts during the first days of liver treatment with fig. III, we find the first event indicated by the double-barbed arrow A. P. pointing in the direction of the macrocyte types of erythroblast. Here we have in a few days a process running counter to that which has lasted for years as the pernicious anaemia has developed. Side by side with this change of the megalocyte types, we must assume there is started an intensive new formation of the pro-macro megaloblasts.

Thus the erythroblast forms to the left in fig. 3 disappear, and the forms to the right become gradually more numerous, i.e. they approach closer to the young, normal erythroblast types, — the macro types. This state of affairs is revealed in the differential counts after sternal puncture in that we find macroblasts in constantly growing numbers, first the younger types, and thereupon the older types (see micro-photo, fig. VI, page 85). When, after a further interval of a few hours, the marrow swings over to the perfectly normal process of macroblast development, the conversion into normoblasts pursues its natural sequence. They become very numerous, and the rise in their number culminates in a peak about the third day synchronously with the peak of the nucleated blood cells from sternal puncture as mentioned in the preceding chapter (see micro-photo V B and VI D page 86 and 88).

These normoblasts in their turn develop through the normal reticulocyte stage into normal erythrocytes.

In this connexion also, the reticulocytes deserve closer attention. DEAN maintains that the reticulocyte crises of pernicious anaemia and the secondary anaemias are completely comparable qualitatively and quantitatively. Here I am in complete agreement with him. In both cases these crises reflect the ability of the bone marrow to form new erythrocytes. But this similarity does not exist before the erythropoiesis of pernicious anaemia has reached the normoblast stage.

COTTI and CIBOLDI find that the average volume of the erythrocytes rises during the crisis to  $150 \text{ my}^3$ , and afterwards goes back to normal. Many others, including myself (see case records) have made the same observation which, according to J. T. BRUGSCH, means that the microcytes disappear and macrocytes appear at the same time in great numbers. WINTROBE maintains that this rise in the number of macrocytes is due to the passage of young and large cells into the blood. COTTI and CIBOLDI believe that this rise is due to the ripening of a great number of megalocyte forms developed from «megaloblasts» of the bone marrow. I do not think that this interpretation of the rise in the average volume of the erythrocytes is correct. As already pointed out in this chapter, it is highly probable that the young erythroblast types, including the megaloblast types, ripen into macroblast-erythroblast types, thereafter passing through the normo types to ripen into normocytes. It is conceivable that a few megaloblast and macroblast erythroblast types may ripen directly into erythrocytes of a somewhat larger type, and that therefore a few of them may be found during a crisis. But this process does not occur on such a scale that it can play any part in the rise of the average volume of the erythrocytes during the crisis. The rise of the erythrocytes in the peripheral blood does not occur till after the reticulocyte crisis in the course of which the following events should be noted:

The rise in the average volume of the erythrocytes during a pernicious anaemia crisis is due to the size of the reticulocytes being somewhat greater than that of normal erythrocytes, and to the fact that, quite temporarily, and on account of the intensive new formations, a certain number of erythrocytes make their appearance and, being some hours younger than is usual, are apt to be somewhat larger in consequence (fig. III). During the crisis one also finds

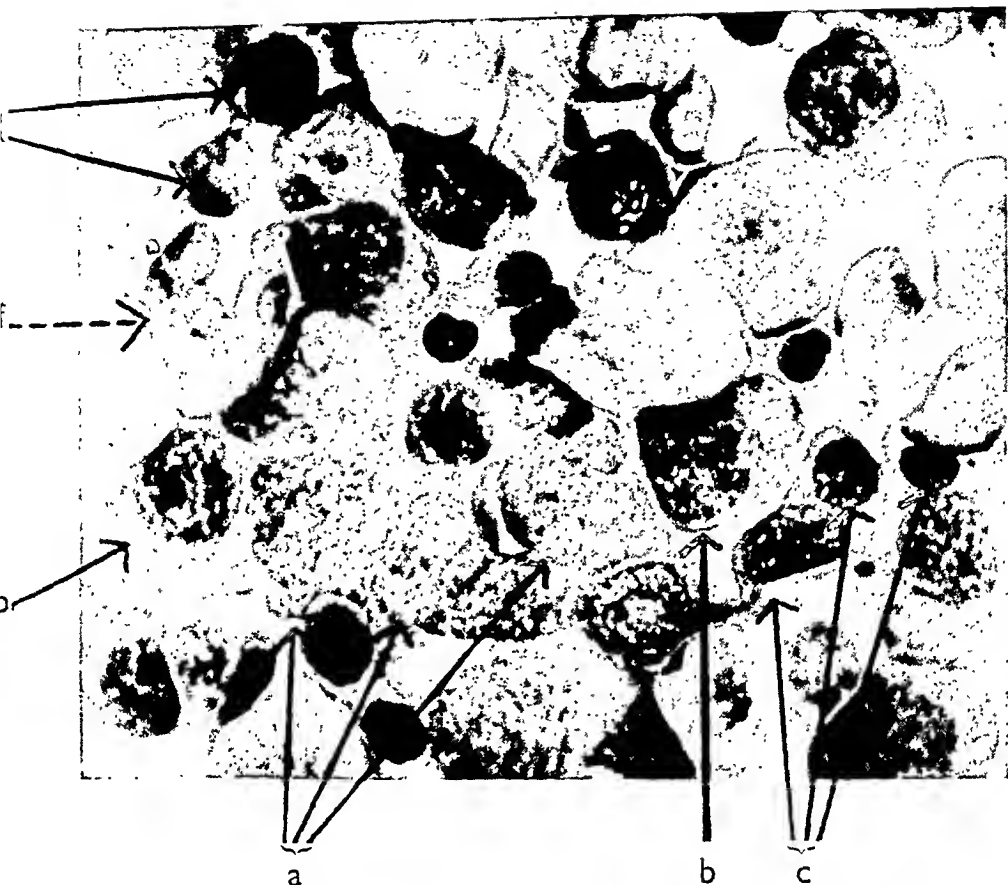
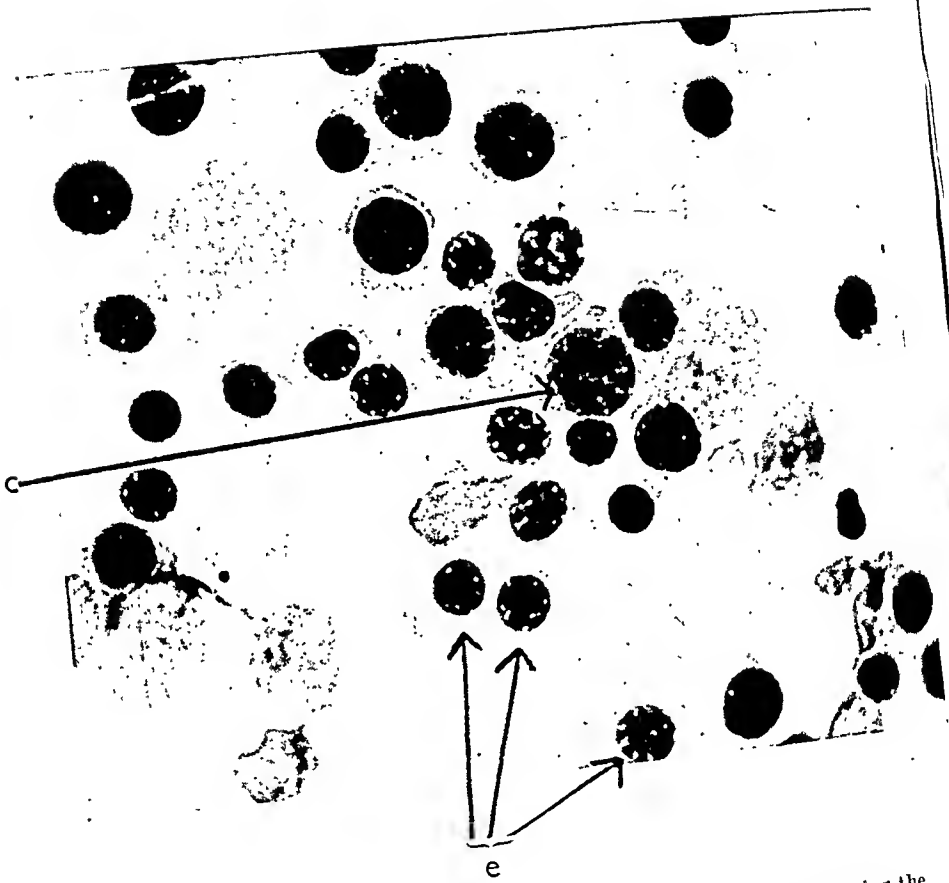
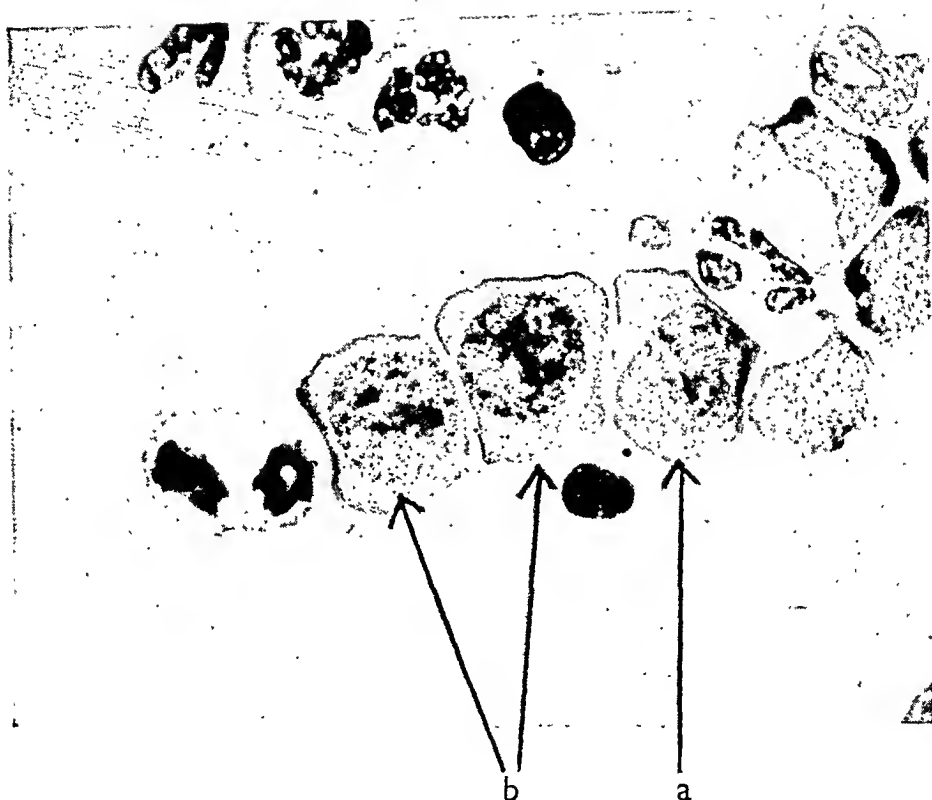


Fig. V. A. Smear (18. 9. 37.) from sternal puncture of untreated case (nr. 36) of pernicious anaemia. There are numerous transitional forms of megakaryoblasts (a) with a somewhat more wide-meshed nuclear structure than that of a typical megakaryoblast, and with remains of the nucleoli of the promegakaryoblasts. Morphologically these cells are more like megakaryoblasts than typical megakaryoblasts. Diameter of nucleus about 15 my. A couple of cells (b) resemble promegakaryoblasts from a developmental point of view and present nucleoli plainly; diameter of nucleus about 12 to 13 my. There are a few (c) more fully developed megakaryoblasts with a denser nuclear structure than the other forms of erythroblast. The nuclei (about 7 to 10 my) are to some extent pyknotic and represent types of erythroblast which will assuredly soon lose their nuclei and ripen into macrocytes. There is also (f) a neutrophile macro-leucocyte, but there are no typical megakaryoblasts in the smear (micro-photo by Dr KAARE HEIBERG. 1000 x enlargement, 1/1 reprod.).

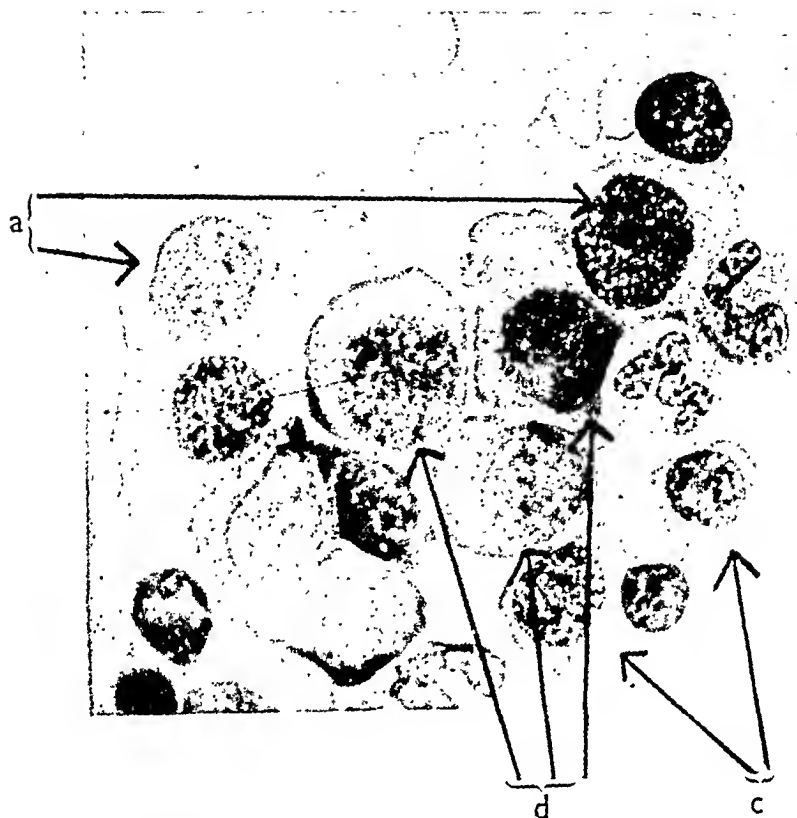


*Fig. V B. Smear (22. 9. 37) from sternal puncture of case nr. 36 showing the basophile phase of the normoblast crisis after liver treatment. Most of the cells (e) are typical basophile normoblasts. diameter of the nucleus about 6--10 my. There are also a few (c) older forms of macroblast, diameter of the nucleus about 11 my. Both these older macroblasts and the normoblasts have a nucleus with a firmly clumped chromatin structure (micro-photo by Dr KAARE HEIBERG, 1000  $\times$  enlargement, 1:1 reprod.).*

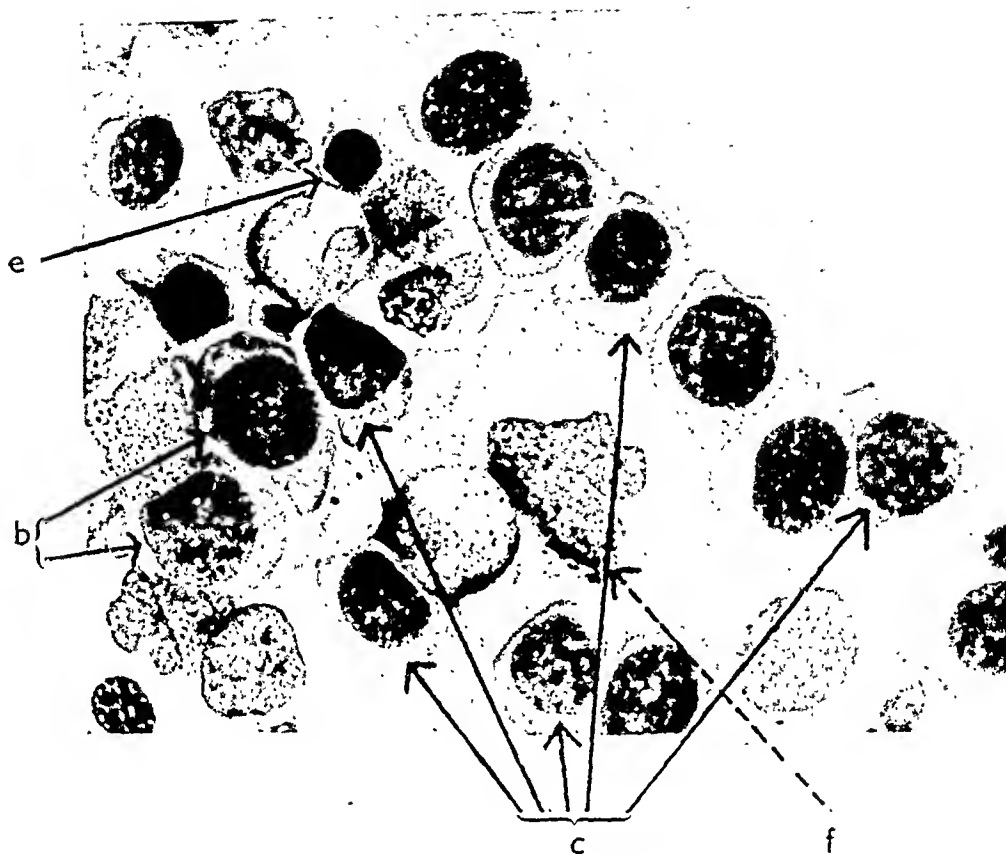


*Fig; VI A. Promacromegaloblasts (b) in sternal puncture smear (8. 2. 37) from case nr. 28, untreated pernicious anaemia. Nucleoli are plainly visible in the nuclei whose structure is somewhat finer than that of the macromegaloblastic transitional forms in fig. V A. They are also plainly a little younger than the promacromegaloblasts in the same preparation, their nuclear structure being also somewhat finer. Diameter of nuclei about 15 my. Beside the two macromegaloblasts is a macromegaloblastic transitional form (a) with a somewhat looser nuclear structure, and with promacromegaloblastic remains of nucleoli. Diameter of nucleus about 15 my This cell resembles the typical megaloblast more closely than the macromegaloblastic transitional forms seen in fig. V A. (microphoto by Dr KAARE HEIBERG, 1000  $\times$  enlargement, 1/1 reprod.).*

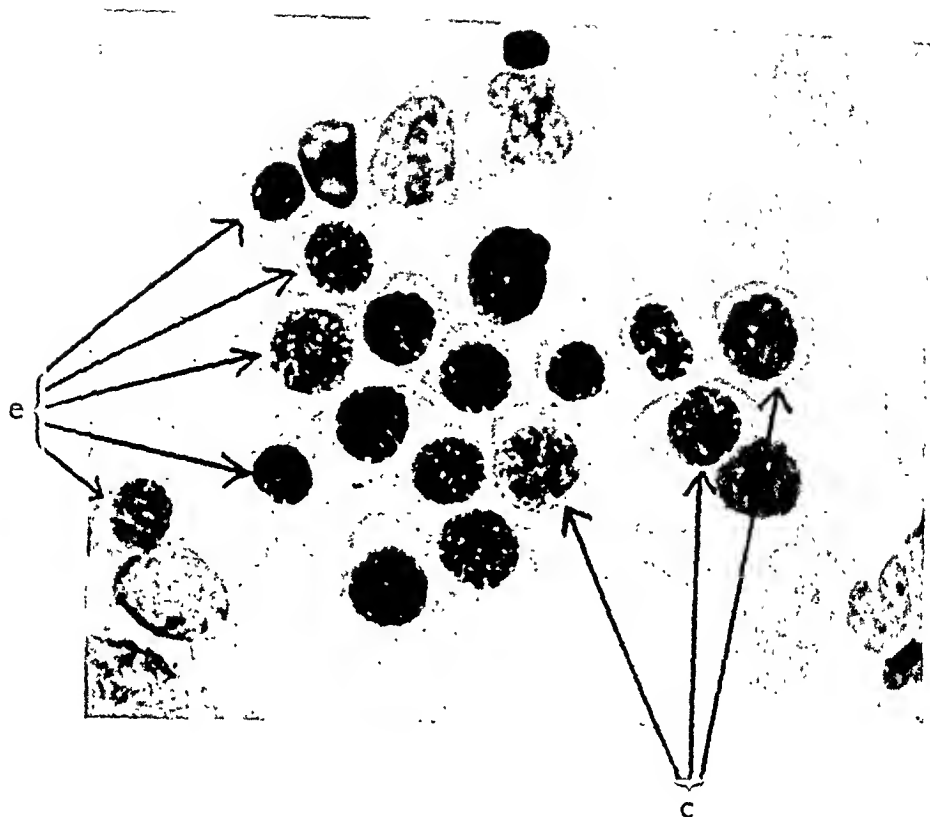




*Fig. VI B. Megaloblasts (d) in sternal puncture smear (8. 2, 37) from case nr. 28. The nuclear structure is finer than in the other erythroblasts photographed in this study. There is only a hint of remains of nucleoli in the nucleus whose diameter is about 15 my. (a) macromegaloblastic transitional forms, (c) macrocytes. (Micro-photo by Dr KAARE HEIBERG, 1000 X enlargement, 1/1 reprod.).*



*Fig. VI. C. Basophile macroblasts in sternal puncture smear (9. 2. 37) from case nr. 28. Young types which have developed during the first 24 hours of liver treatment are seen. Some of the macroblasts are still macromegaloblastic, some are more developed. There are remains of nucleoli. Diameter of nucleus about 10 to 12  $\mu$ . There are a few promacromegaloblasts (b) one normoblast (c) and one macro-leucocyte (f). (Micro-photo by Dr KAARE HEIBERG, 1000  $\times$  enlargement, 1/1 reprod.).*



*Fig. VI D. Smear (10. 2. 37) from sternal puncture of case (nr. 28) on the second day after the injection of a liver preparation. Basophile normoblasts (e). Photo practically identical with that of fig. V B, the only difference being that the former contains a few more erythroblast forms with, perhaps, a slightly finer nuclear structure. These macroblasts seem to be relatively young. Macroblasts (c), normoblasts (e). Diameter of nucleus about 7 to 11  $\mu$ . (Micro-photo by Dr. KAARE HEIBERG 1000 X, enlargement. 1/1 reprod.).*

numerous reticulocytes containing much reticulum and with a hint of «macrocyte characteristics». They are assuredly quite normal normo-reticulocytes which have escaped into the peripheral blood a little earlier than usual and have nothing to do with the megalocytes. Possibly some of them may hail direct from the macroblasts as already pointed out. The rise in the volume of the erythrocytes during a crisis is accentuated numerically by the partial disappearance of the microcytes and the schizocytes, while macrocytes, present before the treatment as more normal erythrocyte forms, still linger for a time in the vascular passages.

CORTI and CIBOLDI also maintain that the reticulocyte crisis of pernicious anaemia is qualitatively different from that of other regenerative processes. It is not likely that this is so, for both processes represent the same, normal regeneration. The only difference is that they *begin* at different stages of the development of erythropoiesis. The regeneration in pernicious anaemia begins with great intensity at somewhat younger, pathological erythroblast stages as already described. In the sternal marrow in other forms of regeneration we find comparatively fewer of these erythroblast forms, but more normoblasts. When the marrow of pernicious anaemia has received enough of the antipernicious anaemia factor and has entered on the normoblastic phase, the modes of regeneration become identified.

### *Conclusion:*

1) *Under liver treatment genuine pernicious anaemia marrow passes through several phases in an orderly way in the course of a few days, the defective, specifically megalomacroblastic marrow becoming ripe.*

2) *The first change is to basophile, young macroblastic marrow.*

3) *The next change is to macro-normoblast marrow (first basophile then eosinophile).*

4) *This starts a normal, physiological reticulocyte crisis.*

5) *The rate of the reaction is constant.*

6) *Coincident with the normoblast crisis in the bone marrow, the normoblasts reach their peak in the peripheral blood.*

7) *During the reticulocyte crisis, the rise in the average volume of the erythrocytes in the peripheral blood depends on the disappearance of the micro- and schizocyte types, numerous young and relatively large*

normo-reticulocytes and a certain number of macrocyte forms making their appearance.

8) When the specific megaloblastic types and the macro-megaloblastic transitional forms of pernicious anaemia are converted to normal macroblasts which ripen into normoblasts, the crisis is morphologically identical with the physiological, erythropoietic processes of regeneration in other conditions.

### C. Treatment with Very Small Doses of Liver Preparations.

*Own investigations.* As mentioned in chapter VI B, the quantity of the liver preparation given does not seem to have much influence on the starting of the typical, primary sternal marrow reactions and of the normal reticulocyte crisis in pernicious anaemia.

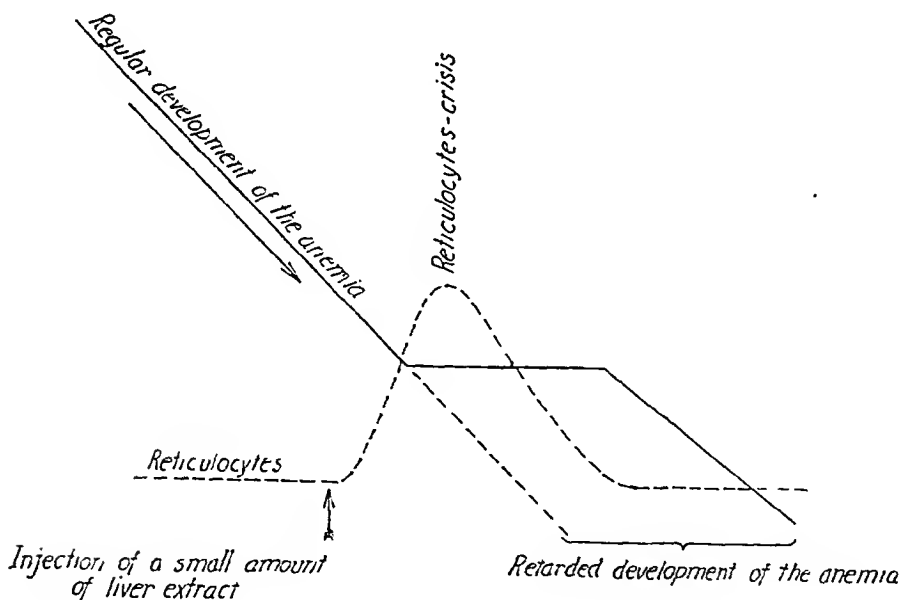
As the case records show, a very small dose was given at the first injection. Table 22 gives the cases in which, in response to liver treatment, the erythrocytes in the peripheral blood rose from 0 to well under  $\frac{1}{2}$  million per  $\text{mm}^3$ . This table also gives the peaks of the rise in the numbers of the reticulocytes and of the erythrocytes in the peripheral blood. The very small doses, it will be seen, provoked a very slight or no rise in the erythrocyte count in the peri-

Table 22.

Casa No.	Reticulocyte peak per thousand	Rise of erythrocytes in the peripheral blood
10	136	from 1.12 mill. to 1.35 mill.
11	264	» 0.70 » » 1.19 »
12	192	» 2.17 » » 2.43 »
13	190	» 2.04 » » 2.22 »
14	168	» 1.19 » » 1.49 »
18	59	» 1.34 » » 1.41 »
19	66	» 1.96 » » 2.03 »
20	.38	» 3.15 » » 3.24 »
23	155	» 1.88 » » 2.16 »
24	159	» 1.88 » » 2.18 »
25	254	» 1.08 » » 1.11 »
26	118	» 1.19 » » 1.30 »
16	95	» 2.20 » » 2.22 »

pheral blood, but they set going the usual bone marrow changes and a practically normal reticulocyte crisis.

Note that in most of these cases liver treatment was followed by the usual reticulocyte crisis in the peripheral blood. But in a couple of cases it was not very marked. From the differential counts of sternal puncture preparations from the cases in this table we can also see, in response to liver treatment, the orderly and regular changes discussed in chapter VI B. But in these cases we do not see the usual rise in the erythrocyte count in the peripheral blood because the dose of liver extract was very small. The sternal marrow gives us a partial solution to this problem, for in some of these cases the usual *total* revolution in it could not be observed. Even after the institution of liver treatment there were still some megalocytes visible in addition to the erythroblast types which give the usual reaction its characteristic feature and which provoke the reticulocyte crisis. Cases 16 and 25, in which the normoblasts had



Fig; VIII. This diagram shows how the injection of a small quantity of a liver preparation provokes a reticulocyte crisis with the characteristic bone marrow changes on which the crisis depends. The new formations are, however, sufficient to check the progress of the anaemia for a time. There is no rise in the number of erythrocytes in the peripheral blood, only stagnation of them (retarded development of anaemia) after which the anaemia again develops as before the injection and with a continued fall of the number of erythrocytes in the peripheral blood.

in a few days again practically disappeared from the sternal marrow, show that the dosage was very cautious. However, as already pointed out, the bone marrow reactions of the patients discussed in this section were practically «normal». Table 17 shows that practically all these patients also reacted with an average rise of some 130,000 nucleated blood cells on sternal puncture, — i.e. the usual reaction at the pernicious anaemia crisis.

How are we to interpret the practically «normal» reticulocyte crisis succeeded by hardly any rise in the number of erythrocytes?

I have attempted to present this problem diagrammatically in fig. VIII which shows the course of events when a small dose of a liver preparation is given in progressive pernicious anaemia. The bone marrow undergoes a certain change and the usual, physiological reticulocyte crisis is started. But owing to the small dosage, the change over of the bone marrow to the normal state lasts a very short time. Only a moderate number of erythrocytes are therefore formed, and they check the progress of the anaemia only for a short time. In response to the changes in the bone marrow towards the normal, there is a simultaneous arrest of the formation of the pathological and small microcytes and schitzocytes with a consequent reduction in the number of erythrocytes in the peripheral blood. This helps partially to hide the small rise in the erythrocyte count which has certainly taken place. As already pointed out, the rate of the reaction of the bone marrow is unchanged.

*Conclusion: A small dose of a liver preparation may be sufficient to start a normal reticulocyte crisis as judged by the usual bone marrow changes. But such a small dose is often sufficient only to put a brake on the progress of the anaemia without effecting an erythrocyte rise in the peripheral blood. The temporary cessation of the formation of microcytes and schizocytes helps to hide the undoubtedly small rise in the erythrocyte count.*

### D. The Influence of Complications on the Effects of Injections of Liver Preparations on the Bone Marrow.

In earlier chapters I have repeatedly touched on the possible effects of complications on the behaviour of liver treatment and the ability of sternal marrow to react to it, and I have maintained that they have little or no effect on the response of pernicious anaemia to this treatment.

*Own investigations.* In the present chapter I have collected all the complications in an attempt to ascertain their frequency and possible significance.

It is sometimes stated, and to some extent taught, that old age seems to have an inhibitory effect on the reaction of the bone marrow. I have therefore in table 23 classified my patients by their ages, in 10-year groups, at the time when their pernicious anaemia was diagnosed.

Table 23.

Age	Number of patients
0—19	1
20—29	1
30—39	7
40—49	10
50—59	13
60—69	16
70—79	13
>80	1

A scrutiny of the case records with regard to a possible relationship between the patient's age and changes in the sternal marrow or a reduced capacity on its part to react, gives no definite indication of any failure in this respect in old age. From the strictly haematological point of view, there was no difference in any other way between the pernicious anaemia of elderly folk and that of younger patients. As many as 14 of my 62 patients were over the age of 70 when their pernicious anaemia was diagnosed. Should age have played any important part, there would thus have been a comparatively greater number of patients reacting sluggishly to the treat-



Table 24. Survey of the character and number of the complications from which the patients in this study suffered.

Complications	Number	Patient, case nr.
Diabetes mellitus .....	5	6—13—17—41—58
Myxodema .....	1	26
Thyreotoxicosis op. ....	1	22
Hypertrofia prostata .....	1	57
Graviditas .....	1	7 Mors
Phlegmonis .....	1	7
Lues congenita .....	1	13
Lues cerebrospinalis .....	1	35
Osteomyelitis tuberculosa .....	1	51
Tbc. gland. colli .....	1	51
Stricture urethra postgonorrhoeica cum haematuria .....	1	15
Cystopyelonephritis haemorrh. ....	1	4 Mors
Pneumonia .....	1	4
Cholecystitis .....	1	55 Mors
Cystopyelonephritis .....	1	55
Rheumatismus acc. ....	1	15
Polyarthrit. chr. ....	2	6—37
Resectio ventriculi .....	2	24—61
Debilitas senilis .....	2	55—61
Hypertensio .....	11	8—22—24—42—48—49— —55—58—61—62—60
Myelosis funicularis grav. ....	5	18—44—59—60—61
Calculi vesicae felleae .....	1	55

ment. My oldest patient was a woman of 82, who died in the Medical Department VII of Ullevaal Hospital of senile debility and general arteriosclerosis complicated by disease of the urinary and biliary tracts and stones in the gall-bladder. Her pernicious anaemia was severe, and she was given liver treatment. Her sternal marrow reacted well with a very satisfactory reticulocyte crisis. Indeed, the erythropoietic reaction was unusually lively, with 900,000 nucleated blood cells per  $\text{mm}^3$ , obtained by sternal puncture on the fifth day after an injection. This was the greatest rise in the number of nucleated blood cells in the whole of my material.

In the histories or clinical observations of most of my patients there was more or less evidence of a lesion of the nervous system.

I could not find that it had the slightest influence on the functions of the bone marrow.

In 27 of my cases I found altogether 42 complications representing 22 different ailments (see table 24). Three of these patients died of their complications, none of pernicious anaemia. Among these 27 patients I could not find any reduction or atypical behaviour of the sternal marrow function with the exception of one case (nr. 4) to be commented on more fully later on.

In this connexion I have again gone through my material with an eye to the possibility of finding cases in which the reaction of the sternal marrow was sluggish or atypical, and I have again come to a stop at case nr. 4.

This patient reacted very badly to liver treatment, and she died six days after admission to hospital, presenting the picture of severe sepsis, with a haemorrhagic and gangrenous cystitis, suppurative pyelonephritis, chronic nephritis, and bilateral pneumonia. The spleen was enlarged. Treatment with liver extract »(X)<sup>c</sup>-benz», had not the slightest effect on the sternal marrow. This preparation proved very effective in several other cases. One may therefore conclude that severe sepsis may reduce the effect of liver-injection treatment either because the bone marrow is paralysed or the preparation is hindered in some way or other from acting.

This was the only patient in my material of whom it could be said with some degree of certainty that a complication hindered the action of liver of bone marrow.

Further, the reaction of the sternal marrow to treatment was less lively than usual in cases 15, 18, 44, 62 and 20, in all of which at the commencement of liver treatment the anaemia had reached a comparatively moderate stage, and the sternal marrow contained some normoblasts. As already pointed out, the changes found on sternal puncture under such conditions are not massive, when the marrow is not completely converted into the »typical pernicious marrow». To be sure, the erythrocyte count in the peripheral blood rises all the same in a satisfactory way in response to treatment. Consequently, the reaction of the sternal marrow was normal and satisfactory in these cases, and it did not betray any defect.

*Conclusion: Apart from a case which was complicated by severe*

*and fatal sepsis and which reacted badly to liver treatment, I have found nothing to show that complications, whatever their character, reduce the activity of the bone marrow or the action of liver treatment. Nor does there seem to be any demonstrable reduction in the activity of the marrow in old age.*

### E. The Second Treatment in the Same Regeneration Period.

*Own investigations.* In four cases (nrs. 13, 25, 32 and 43) I have had occasion to investigate by sternal puncture the action of liver treatment for the second time in the same regeneration period.

In case 13, the second treatment was followed by a complete crisis, the findings of sternal puncture, from a purely morphological point of view, were identical on the two occasions. This was also so in case 25, and in both cases the normoblasts had practically disappeared from the sternal marrow after the first injection when the second injection was given.

In case 32, the first injection was followed by a marked sternal marrow reaction, and in the peripheral blood the erythrocytes rose from 1.42 to 2.73 million per  $\text{mm}^3$ , at which stage the second injection was given. There were now more than 60 per cent, normoblasts on sternal puncture. A satisfactory and definite reaction followed the second injection also, with the development of new normoblasts, and thus it was possible to follow a new basophile and eosinophile normoblast crisis. The reaction was, however, definitely toned down, — a feature observed earlier in this study when liver treatment was given at a normoblast stage in the marrow.

Patient nr. 43 was admitted to hospital during a lively regenerative crisis. Fourteen days later, when a liver extract was injected, no normoblasts could be found in the sternal marrow and, as was to be expected, the injection started the usually lively regenerative crisis.

These four cases indicate, as was anticipated, that the reaction to liver treatment is the same whether the patient has or has not been treated earlier. When there are numerous normoblasts on sternal puncture, the reactions already described are moderate, whereas

they are well marked when the normoblasts have again disappeared after the first injection.

*Conclusion: On a second injection of liver during the same regeneration period, the sternal marrow reacts in principle in the same way as in cases of untreated pernicious anaemia. This reaction is more and more toned down as the number of normoblasts rises. This also happens in untreated cases of pernicious anaemia.*

## VIII. The Sternal Marrow after Recovery.

As already pointed out, and in the opinion of every author, the sternal marrow becomes perfectly normal on the patient's recovery from pernicious anaemia. My own observations support this statement.

Table 25 gives the collected findings of nucleated blood cell counts, and the percentage interrelationship of the erythropoietic and leucopoietic cells in the sternal marrow. These investigations (second column) were undertaken a comparatively long time after liver treatment had been started and after the anaemia (first column) had receded markedly.

In the fourth column of table 25 are the figures for nucleated blood cells yielded by sternal puncture, their average number being 76,800 per  $\text{mm}^3$ . This figure is close to the 98,000 found in my own normal material. Isolated figures are also within the range of variation of my normal material. This is also the case with the interrelationship of erythropoiesis and leucopoiesis. The third column of table 25 gives the average percentage of erythroblasts as 18.7, — a figure which tallies completely with my normal findings. The isolated figures for the percentage of erythroblasts are also within the range of variation of my normal figures.

Table 26 gives the differential counts of smears from the sternal puncture of patients dealt with in this section. A comparison of these figures with those of my differential counts of normal material (table 2) again shows completely parallel figures. In a few cases the macroblast figures are rather high. This is not surprising as the erythropoiesis in my material was not fully stabilized at a normal level by adequate treatment for a considerable time.

Table 25. A survey of the number of nucleated blood cells and of the percentage of erythroblasts among them from cases given liver treatment for a considerable time.

Case nr.	Number of erythrocytes in ear-blood in millions	Day after injection	Erythroblasts per cent.	Number of nucleated blood corpuscles in sternal puncture
3	3.23	58	15.4	39,000
11	4.58	s.t.	13.8	37,100
17	3.31	21	17.5	30,100
"	3.79	37	22.7	12,800
21	3.32	8	25.—	23,500
25	3.37	31	23.4	49,200
28	4.35	52	19.7	45,600
33	3.72	66	13.2	37,000
39	4.50	s.t.	12.4	—
42	4.70	s.t.	27.6	105,000
46	4.12	70	19.1	204,500
51	3.43	25	15.9	—
52	3.83	43	11.4	—
56	1.92	16	19.5	120,000
61	2.65	15	24.1	218,000
62	4.10	17	18.1	—
Average			18.7	76,800

s.t.: sufficient therapy for years.

*Conclusion: Under liver treatment, the sternal marrow yielded by sternal puncture changes in a short time, both quantitatively and qualitatively, so that it becomes absolutely identical with the normal sternal marrow yielded by sternal puncture.*



## IX. The Serum Colour in Relation to the Sternal Marrow Findings in Pernicious Anaemia.

As early as 1918, NÆGELI maintained that »haemolysis» is of little importance in pernicious anaemia. and that the roots of the disease are to be sought in the primary bone marrow changes. But such well known investigators as PAPPENHEIM and MORAWITZ have regarded primary haemolysis as the essential factor in the development of the anaemia. More recent research (by TEMPKA and BRAUN for example) has failed to give a definite answer to this question.

In this chapter I do not propose to deal in greater detail with the increased bilirubin content of the blood nor with the enormous literature of this subject. Here I wish quite briefly to show that there is no relationship between the degree of the raised serum colour, the findings of sternal puncture, and the degree of the anaemia.

Table 27 gives from above downwards the findings from various patients according to the extent to which the serum colour is increased in untreated pernicious anaemia. The second column, dealing with the serum colour, shows no higher figure than 20. The third column gives the corresponding degree of the anaemia. Here we can find no parallel behaviour, no correlation between the recorded figures. The two patients lowest in the table (nrs. 54 and 57) for example, suffered from severe anaemia, but showed no increase of the serum colour in spite of the fact that the differential count from smears yielded by sternal puncture (columns 5, 6, 7) showed a marked relative displacement towards the erythropoietic types of cell. In these smears there were no normoblasts, but a great number of megaloblast and macro-megaloblast transitional forms of the erythroblasts. The converse state of affairs in case nr. 17, for



Table 27. A survey of the serum colour in relation to the degree of the anaemia, the number of nucleated blood cells of the sternal marrow, and the morphology of erythropoiesis in untreated pernicious anaemia.

Case nr.	Serum colour	Anaemia in millions of erythrocytes	Number of nucleated cells in sternal blood	Erythroblasts per cent in sternal blood	Erythrobl. in sternal-blood	
					Megalo-blasts per cent	Normo-blasts per cent
56	20	1.03	137,800	54.89	4.50	0
17	18	2.50	64,800	30.40	3.50	63.0
34	18	2.00	77,600	57.80	14.00	0
47	17	0.97	35,000	33.50	5.00	0
51	17	1.41	71,400	46.90	15.00	0
59	17	1.65	62,600	39.20	6.50	0
28	14	1.40	105,900	19.70	16.00	0
29	14	2.46	240,000	61.30	15.00	0
11	14	0.69	43,000	43.60	4.60	0.5
58	14	1.43	57,900	53.90	23.50	0
21	13	2.60	43,000	23.90	3.00	25.0
31	13	2.38	9,100	29.00	0.00	45.0
52	13	1.14	110,400	54.90	13.00	0
10	12	1.12	71,000	47.60	5.50	0
30	12	1.20	—	30.20	15.00	0
48	12	1.59	105,600	38.80	3.70	0
1	11	1.39	87,800	27.00	4.00	0
8	11	2.13	50,000	46.90	7.00	1.0
55	11	2.01	76,000	33.30	13.50	0
5	10	2.77	64,600	23.00	13.00	3.5
26	10	1.10	129,000	38.00	11.50	0
50	10	1.04	67,000	54.50	14.50	0
62	9	2.56	41,800	23.20	0.00	34.0
38	9	0.91	101,500	44.10	5.00	0
25	9	1.18	101,500	37.50	9.50	0
23	9	1.88	72,900	32.90	8.50	0
14	9	1.19	74,800	38.00	11.60	0
6	9	1.67	191,000	52.60	8.00	0
4	9	0.89	61,800	35.50	5.50	0
13	8	2.13	123,700	34.60	9.20	0
20	8	2.80	30,900	16.00	5.00	13.0
24	8	1.88	55,700	44.10	5.00	0
32	8	1.37	98,000	35.00	19.40	0
36	8	0.95	64,200	48.50	2.00	0
46	8	2.24	129,500	23.90	4.50	7.0
2	7	1.53	51,000	45.80	1.00	0
3	7	4.58	39,000	15.40	3.90	47.0
12	7	2.17	21,800	26.80	12.00	0
18	7	1.37	64,300	40.40	6.40	0
35	7	2.27	42,100	23.60	19.70	7.6
45	7	2.59	28,200	25.80	5.00	33.0
53	7	1.16	138,800	49.90	17.00	0
49	6	1.89	116,400	44.50	9.50	1.0
27	6	1.58	—	22.00	10.00	0
18	6	1.11	226,300	55.20	5.00	0
19	6	1.96	191,900	35.00	12.00	0
16	6	2.20	66,000	22.90	5.00	17.0
61	5	2.25	154,000	38.70	5.50	0
41	5	3.39	86,100	26.30	—	36.0
33	5	1.33	208,100	50.90	10.50	0
7	4.5	1.72	68,900	37.50	10.50	0
9	4	3.28	57,800	16.80	—	76.0
15	4	2.38	44,900	24.00	2.60	44.0
60	4	1.78	96,000	—	—	0
37	3.5	4.50	—	12.40	—	91.0
37	3	2.69	104,800	32.00	11.40	16.2
54	3.5	1.03	96,000	39.80	10.00	0
57	2	1.40	93,200	41.70	16.50	0

example, was found, with a relatively much-increased serum colour (to 18). The table shows that this patient suffered from a comparatively moderate degree of anaemia with a profusion of normoblasts. The case records show that the patients' ages had nothing to do with this lack of conformity.

*Conclusion: The height of the serum colour and the degree of the anaemia show no parallel behaviour, and there is therefore, as already demonstrated, no correlation to be established between the height of the serum colour and the changes found on sternal puncture. The patients' age is of no importance in connexion with this lack of conformity.*

## X. Summary.

Out of the jumble of cell forms described and from the diversified nomenclature in the literature on the cell types of erythropoiesis, I have selected and described certain main types to which, according to a simplified nomenclature, are given the names: promacro-megaloblasts, megaloblasts, macroblasts and normoblasts. A detailed description of the morphology of these cells is given. The megaloblast types, assumed to be more vulnerable than the other types of erythroblast, are pathognomonic of failure of the antipernicious anaemia factor. The precursors of *all* the erythrocyte types are developed from the same parent cells (promacro-megaloblasts) and undergo the same erythroblastic developmental changes which may vary somewhat morphologically if there is a shortage of the substances necessary for normal cell development (see, for example, the megaloblast types). The teaching that there are various so-called developmental series is abandoned, and it is maintained that all the forms of erythroblast mentioned give rise to all of the known forms and types of erythrocyte (see fig. III).

In pernicious anaemia, the large erythrocytes found in the peripheral blood are mainly derived from the bone marrow's macroblastic and macro-megaloblastic transitional forms, and should be described as macrocytes. In their most typical form, the megaloblasts do not give rise to erythrocytes (megalocytes), but degenerate at an early stage. The oval shape of certain erythrocytes is taken to show they are relatively old.

Table 2 gives my own sternal puncture findings in normal subjects. After a critical perusal of the corresponding investigations of other observers, I have found the results to tally completely, and to do so also in respect of my own patients with untreated pernicious anaemia. This concordance is so striking that I believe I

am completely justified in maintaining that the findings of sternal puncture give an excellent picture of the quantitative and qualitative activities of the sternal marrow. Sternal puncture also gives an unequivocal picture of the bone marrow and its cells. Changes in the marrow are instantly reflected, morphologically and numerically, in corresponding changes in the yield of sternal puncture.

Occasionally, macroscopic and normally pure foci of fat are found in the sternal marrow in which both haematopoietic tissue and fat are developed in abundance. Sternal puncture yields a somewhat higher proportion of fatty tissue than is found in the bone marrow possibly because fat may be more easily aspirated than normal cell marrow whose aspiration provokes a moderate degree of pain.

Sternal puncture of 49 patients with untreated pernicious anaemia showed a relatively very moderate number of nucleated blood cells if account is taken of the severity of the anaemia. The average figures for these cases were below the average figures under normal conditions, and isolated counts were within the normal range of variation. These counts showed a slight tendency to rise as the anaemia progressed, a slight increase in the number of constantly younger, nucleated erythropoietic cells being responsible for this rise. During the first period of their development, the nucleated blood cells in the marrow of pernicious anaemia were less numerous than normal, and it would therefore seem that the initial stage of the anaemia is characterized by a more moderate development than normal of myeloid cells, both erythropoietic and leucopoietic. As the anaemia progresses, the erythroblasts are arrested in their development at an early stage and undergo to a certain extent megaloblastic degeneration because the process of maturing is inhibited. Some of them are gradually stored up as such with the result that their numbers rise again somewhat. With the further development of the pernicious anaemia, the percentage of erythroblasts on sternal puncture rises above normal, and this may mean that they are being stored up because their further development is arrested by the failure of the supply of the antipernicious anaemia factor. As the cell count on sternal puncture does not fall with the progress of the anaemia, it is highly probable

that there is some rise, *of a very moderate degree*, both absolute and relative, in the number of the erythroblasts, while there is a definite and constant dwindling of the numbers of the leucopoietic cells.

In untreated pernicious anaemia, aspiration of sternal marrow provokes much more pain than that of normal sternal marrow. In this disease sternal puncture yields abnormally red marrow containing exceptionally numerous particles of marrow whereas there is at the same time a considerable reduction in the number of fat particles. These changes become more marked as the anaemia progresses, but in a few cases foci of fat may continue to be found. A »normal» agonal disappearance of the cells of the marrow, and a growth of fatty tissue may possibly occur in pure pernicious anaemia. A yield on sternal puncture of a relatively considerable quantity of fat when the anaemia is moderate may be due to some of the normal fatty tissue having remained in place. In a few cases of pernicious anaemia the function of the sternal marrow may be unusually lively.

In untreated pernicious anaemia, sternal puncture reveals the development of characteristic morphological changes in the erythropoiesis. They fall naturally into three stages, — the initial, normomacroblastic stage, the normo-macromegaloblastic intermediate stage, and the macromegaloblastic final stage (III). This last stage is usually reached before the effect of the anaemia drives the patient to seek medical advice.

Megaloblasts are not demonstrable in normal sternal marrow, and this form of erythroblast does not appear on sternal puncture before the pernicious anaemia has reduced the erythrocytes in the peripheral blood to fully three million per  $\text{mm}^3$ . The promacromegaloblasts become at the same time more numerous, reaching their peak together with the typical megaloblasts when the anaemia has fallen below two million erythrocytes. At the same time the number of macroblast cells rises evenly with the progress of the anaemia, from less than the normal 10 per cent. on sternal puncture to over 80 per cent. when the pernicious anaemia is fully developed. Simultaneously with their rise (which may be slight in absolute figures and is definite relatively) the macroblasts change from the morphologically normal and somewhat older type to almost exclusively macromegaloblast transitional forms when the anaemia is

fully developed. It is this type of cell which predominates on sternal puncture. As the anaemia progresses, the normoblasts disappear, and they are not demonstrable when the erythrocytes in the peripheral blood fall below two million. This rule is so absolute that when the sternal marrow yields normoblasts in untreated anaemia with less than two million erythrocytes, the diagnosis of pernicious anaemia must be rejected. The presence of numerous pyknotic erythroblasts could be indicative of a complication, probably toxic, such as chronic polyarthritis, congenital syphilis or chronic enterocolitis.

The leucocyte types of cell yielded by sternal puncture also change with the development of pernicious anaemia. The granulocytes and their precursors remain numerically unchanged in relation to each other. But they change morphologically, and we find larger varieties of them as with the erythropoietic cells. These larger cells have been called macroleucocytes. There is an increase in the number of reticulo-endothelial phagocytic elements, and this increase may be due to the abnormal metabolism in association with the pathological erythropoiesis. There is also a rise of the percentage of macerated cells partly on account of the pathological erythropoiesis, the megalocytes being more vulnerable than the other erythropoietic cell types. The megacaryocytes remain unchanged during the progress of pernicious anaemia, while the percentage of lymphocytes falls, the fall being to some extent relative.

The author cannot accept the view generally held that the bone marrow of pernicious anaemia is hyperplastic and hyperactive.

The author has shown that the number of nucleated blood cells yielded by sternal puncture is increased only to a very doubtful extent in the final stage of pernicious anaemia, whereas earlier in the disease it seems to be reduced. The volume of these cells is, however, much increased. It seems to him that the red and somewhat enlarged bone marrow gives an impression of «aregenerative», to some extent degenerative hypertrophy. Its functional capacity is reduced, and it is neither hyperplastic nor hyperactive. This is how the author visualises the development of pernicious anaemia:

The supply of the antipernicious anaemia factor fails, and the bone marrow, which had been normal in extent and function, ceases to work as before. The cells change and hypertrophy, the marrow

becoming inactive. We now find in the haematopoietic system a primary hypertrophy in its normal area of distribution in which the fatty marrow is displaced. The reduced functional activity of the marrow leads to incipient anaemia and granulocytopenia which stimulate the haematopoietic properties of the organism now producing all it can of haematopoietic tissue. This happens throughout the bone marrow which becomes hyperplastic, consisting to a large extent of young erythropoietic cells. This happens also on the administration of a small amount of the antipernicious anaemia factor with the result that the erythroblasts are arrested in their development at an early stage. The result is a hypertrophic bone marrow, functionally inactive, and with exactly the same character as the bone marrow which develops in those areas in which one normally finds actively functioning, haematopoietic cell marrow. The whole of the haematopoietic cell marrow of the bony system is now hypertrophic and inactive.

The reticulocyte counts in the peripheral blood and from normal sternal puncture are under the normal upper limit of 20 per thousand in normal blood. In pernicious anaemia, the reticulocyte count is somewhat higher than normal, more so on sternal puncture than in the peripheral blood. But in relation to the degree of the anaemia and the hypertrophy of the erythropoietic tissues of the bone marrow, the rise of the reticulocyte count is so moderate that this also points to the productive capacity of the bone marrow being small.

In response to liver treatment, the number of nucleated blood cells yielded by sternal puncture rises above the normal range of variations. This happens invariably, and the peak is reached about the third day. At this stage the bone marrow is to be regarded as hyperplastic and hyperactive from the anatomical and functional points of view. Provided there is not a high percentage of normoblasts in the bone marrow, this reaction is practically independent of the degree of the anaemia when liver treatment was started. *To set this reaction going*, the dosage of the injected liver preparation seems to be of little importance. Also in response to liver treatment, there is a marked rise in the percentage of the erythroblasts in relation to the nucleated blood cells yielded by sternal puncture. This rise of the erythroblasts runs parallel with the rise

in the number of nucleated blood cells, and both reach their peaks about the third day. The extent of the reaction is not, however, so great that one can discount the possibility that the increased cell count on sternal puncture also depends, in a much more moderate degree, on a rise of the leucocyte types of cell. Also in this matter the quantity of the liver preparation injected and the degree of the anaemia at the time of the injection seem to be of minor importance. It is, however, conceivable that in pernicious anaemia, the extent of the reactions mentioned may be somewhat less marked, and that the rise in the percentage of erythroblasts is somewhat greater when the erythrocytes in the peripheral blood rise higher than usual after liver treatment.

According to the degree of failure of the antipernicious anaemia factor, we must be prepared to find pernicious anaemia running its course in different ways.

Corresponding to the purely quantitative changes in the yield of sternal puncture there are perfectly rule-bound qualitative changes in response to liver treatment within 24 hours of which the first changes are already to be observed in most cases. The cells of the megalomacroblastic type in the sternal marrow change, developing (ripening) in the direction of normal erythroblast forms. After about 24 hours the marrow is basophile and young-macroblastic. On the second day it is basophile and macro-normoblastic, and about the third day it is eosinophile and normoblastic, and this stage coincides with the highest count of nucleated blood cells found on sternal puncture. Following these changes in perfectly rule-bound fashion is the erythroblast crisis with peaks on one of the closely ensuing days. The erythroblast peak in the peripheral blood coincides with the erythroblast peak in the sternal marrow about the third day, and like it is an eosinophile normoblast crisis. The reaction seems to proceed somewhat more sluggishly if the liver treatment is given early in the development of the anaemia while there are still many normoblasts in the bone marrow, or if there is a serious complication of a toxic character.

When the megaloblasts and the megalomacroblastic transitional types have been transformed, the subsequent reaction of the bone marrow is identical with the physiological regenerative erythroblast reaction.



A study of the erythroblast types during regeneration shows that the megalocytes develop through macroblastic stages into normal normoblasts which in their turn mature further to erythrocytes of normal type, — normocytes.

The increase in the average volume of the erythrocytes shortly after liver treatment is started is due to the fact that the young reticulocyte is a little larger than the mature erythrocyte. By the numerical standard alone, this phenomenon is accentuated by the gradual disappearance of the microcytes and schizocytes when the bone marrow function returns to normal, whereas the macrocytes live on some time longer as more normal types of erythrocyte. Any possible new formation of macrocyte is so small that it may be assumed to be without any demonstrable effect on this rise in volume.

A small dose of liver extract can start a normal reticulocyte crisis without any subsequent demonstrable rise of the erythrocytes in the peripheral blood. This is so because the dose is large enough only to check the progress of the pernicious anaemia by restoring the bone marrow to normal for quite a short time. The small erythrocyte rise, undoubtedly taking place, is also obscured by the behaviour of the bone marrow which, acting normally for a short time, arrests the further pathological development of microcytes and schizocytes with the result that they are to a certain extent and temporarily absent from the peripheral blood. Their relatively great numbers may have this effect that the newly formed normocytes are not numerous enough to hide the numerical deficiency of the pathological erythrocytes.

As a rule, complications of various kinds do not seem to inhibit the functions of the sternal marrow nor the action of liver treatment. But in a case of sepsis running a fatal course, the functions of the marrow did seem to be paralysed or, perhaps, the effect of the liver treatment was shortened. Old age seems to have no demonstrable effect on the functions of the bone marrow in pernicious anaemia.

When the treatment is given a second time during the same regeneration period, the sternal marrow reacts in principle to it in the same way as in untreated pernicious anaemia. But if the second treatment is given relatively soon after the first, the reaction is

somewhat toned down, particularly if the normoblast count on sternal puncture is high.

Under adequate liver treatment the bone marrow again becomes perfectly normal, and the yield of sternal puncture is indistinguishable from the normal.

If we compare the sternal marrow changes observed in this study of untreated cases of pernicious anaemia with the behaviour of the serum colour of the blood, we find no demonstrable relationship between its level and the degree of the anaemia. I have also failed, naturally enough, to find any relationship between the level of the serum colour and the degree of the purely quantitative and qualitative changes in the yield of sternal puncture.

Table 28. Differential counts of sternal

Pat. nr.			2	3	4	5	6	7	8	9
Sternal puncture			Asp. easy 0.3 cc. st. blood m.p. +++ f.p. +++ moderate a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ f.p. +++ moderate a. pain	Asp. easy 0.35 cc. st. blood m.p. +++ f.p. +++ no a. pain	Asp. easy 0.3 cc. st. blood m.p. +++ f.p. +++ no a. pain	Asp. under vac. (5 cc.) 0.1 cc. st. blood m.p. ++ f.p. ++	Asp. easy 0.15 cc. st. blood m.p. +++ f.p. +++ moderate a. pain	Asp. under vac. (10 cc.) 0.3 cc. st. blood m.p. ++ f.p. ++ no a. pain	Asp. easy 0.15 cc. st. blood m.p. +++ f.p. +++ moderate a. pain
Erythropoiesis	Erythroblasts	per cent	21.60	20.10	18.10	10.90	20.30	18.90	12.50	16.10
	Leuco- blasts	"	78.40	79.90	81.90	89.10	79.70	81.10	87.50	83.50
	cytes	"								
	Pro- megalobl.	"	—	1.—	2.—	—	2.—	1.—	1.—	1.—
	Macrobl. bas.	"	—	—	—	—	—	—	—	—
	Macrobl. eos.	"	—	—	—	—	—	—	—	—
	Macrobl. bas.	"	4.—	5.—	6.—	3.—	9.—	16.—	—	8.—
	Macrobl. eos.	"	1.—	—	—	—	2.—	—	—	—
	Normobl. bas.	"	56.—	74.—	59.—	69.—	61.—	60.—	89.—	65.—
	Normobl. eos.	"	36.—	18.—	30.—	26.—	25.—	23.—	10.—	19.—
Leucopoiesis	Erythrobl. bas.	"	1.—	—	—	—	—	—	—	—
	Erythrobl. eos.	"	—	—	—	—	1.—	—	—	—
	Erythrobl. bas.	"	1.—	2.—	3.—	2.—	—	—	—	3.—
	divisions forms eos.	"	1.—	—	—	—	—	—	—	3.—
	Meganearyocytes	"	—	—	0.25	—	—	—	—	—
	Mast immature	"	0.25	0.25	0.25	—	—	—	0.25	0.25
	cells mature	"	—	—	0.25	—	—	—	0.25	—
	Eos. myelocytes	"	1.50	2.75	1.75	0.75	2.25	2.75	0.25	1.—
	Leuco- band forms	"	1.25	2.75	1.75	1.50	1.—	2.—	0.75	0.50
	cytes polymorphs	"	0.50	0.75	0.75	0.75	0.75	1.—	0.50	0.50
	Myeloblasts	"	2.75	4.—	1.50	2.50	1.75	1.25	1.50	1.75
	Praemyelocytes	"	2.—	4.75	5.50	4.50	4.25	4.—	3.50	8.—
	myelocytes	"	17.75	22.25	12.50	13.—	23.50	19.75	9.25	13.50
	Neu- young forms	"	30.50	28.25	30.25	29.75	33.50	30.50	25.75	27.50
	philes band forms	"	14.50	10.50	11.—	15.25	9.50	8.25	16.50	8.50
	philes polymorphs	"	6.50	6.75	13.—	7.75	6.50	11.—	17.25	7.75
	Mono- blasts	"	1.25	3.50	0.75	1.25	0.50	1.25	2.25	1.25
	cytes	"								
	Lymphocytes	"	15.50	11.—	13.75	20.50	11.50	10.75	20.75	15.50
	Plasma & Türk cells	"	1.50	2.—	1.50	0.50	—	0.75	—	1.75
	Smear cells	"	4.—	3.—	5.25	1.75	5.—	6.50	1.25	7.—
	Reticuloendothelial cells	"	0.25	0.50	—	0.25	—	0.25	—	0.25
	Nucleated blood cells	"	115 200	127 800	92 300	39 300	51 400	148 500	204 400	131 700
Reticuloocytes per mill.			—	3	2	8	15	2	8	5

puncture smears from own normal material.

10	11	12 A	12 B	13	14	15	16	17	18	19	20	21
Asp. easy 0.2 cc. st. blood m.p. +++ f.p. +++ + severe a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ f.p. +++ + moderate a. pain	Asp. under vac. (10 cc.) 0.1 cc. st. blood m.p. 0 f.p. +++ + no a. pain	Asp. under vac. 0.2 cc. st. blood m.p. traces f.p. +++ some a. pain	Asp. under vac. (15 cc.) 0.5 cc. ab. blood m.p. +++ + f.p. +++ + some a. pain	Asp. under max. vac. (20 cc.) 0.1 cc. st. blood m.p. (+) f.p. +++ no a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ + f.p. +++ + severe a. pain	Asp. under vac. 0.2 cc. st. blood m.p. +++ + f.p. +++ + moderate a. pain	Asp. easy 0.2 cc. st. blood m.p. +++ + f.p. +++ + moderate a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ + f.p. +++ + moderate a. pain	Asp. easy 0.15 cc. st. blood m.p. +++ + f.p. +++ + moderate a. pain	Asp. easy 0.5 cc. st. blood m.p. +++ + f.p. +++ + some a. pain	Asp. under vac. (15 cc.) 0.15 cc. st. blood m.p. (+) f.p. +++ no a. pains
20.90	17.10	6.—	9.30	16.50	12.50	19.50	14.50	13.10	22.50	14.50	15.90	11.50
79.10	82.90	94.—	90.70	83.50	87.50	80.50	85.50	86.90	77.50	85.50	84.10	88.50
3.—	—	—	—	1.—	—	—	—	—	1.—	1.—	—	2.—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
9.—	5.—	7.—	6.—	11.—	7.—	6.—	4.—	9.—	7.—	7.—	7.—	4.—
—	—	—	1.—	—	—	—	—	—	—	—	—	3.—
74.—	81.—	41.—	74.—	73.—	86.—	79.—	76.—	75.—	72.—	83.—	78.—	67.—
8.—	12.—	12.—	17.—	14.—	6.—	11.—	17.—	9.—	18.—	9.—	12.—	20.—
3.—	—	32.—	—	—	—	3.—	1.—	—	—	—	—	—
—	—	8.—	2.—	—	—	—	—	—	—	—	—	—
2.—	2.—	—	—	1.—	1.—	1.—	2.—	3.—	2.—	—	2.—	3.—
1.—	—	—	—	—	—	—	—	—	—	—	1.—	1.—
—	—	—	0.25	0.25	0.25	—	0.75	—	—	0.75	—	0.25
0.50	—	—	—	—	0.25	0.25	0.25	—	0.50	0.25	0.25	0.25
—	—	—	—	—	0.25	—	0.25	—	—	—	0.75	—
0.50	0.50	0.25	1.75	0.50	1.25	1.75	1.—	2.75	3.75	1.—	1.75	2.25
0.25	0.25	1.25	1.50	1.25	1.50	1.25	1.—	2.50	2.50	1.—	1.25	1.50
0.25	—	2.25	1.—	0.50	1.75	0.50	0.50	1.25	1.50	1.—	0.50	0.25
0.50	2.25	1.—	1.50	2.25	1.—	3.—	4.25	1.25	4.50	3.—	2.50	1.25
4.—	2.50	2.—	1.50	3.25	5.—	9.—	6.25	6.75	5.75	9.75	3.—	4.25
14.50	13.25	4.50	11.—	12.75	13.75	15.—	14.25	13.50	12.—	15.—	8.75	12.25
29.—	37.50	9.—	26.75	31.75	33.—	22.25	25.—	31.—	25.75	29.50	28.50	37.25
16.50	9.—	7.25	9.75	11.75	12.75	10.—	16.—	11.50	13.75	11.25	12.50	12.25
14.50	4.75	24.—	17.—	9.—	9.25	9.50	5.25	9.50	10.25	5.75	12.25	17.—
1.25	1.50	4.25	2.—	3.25	1.75	2.—	2.—	1.—	2.—	2.25	2.—	0.50
14.75	24.—	42.—	18.25	18.75	16.50	17.75	16.50	16.25	13.75	15.—	20.25	9.25
2.75	0.50	—	0.75	—	—	2.25	1.25	0.25	1.—	1.25	2.—	0.25
1.75	4.—	2.25	7.—	4.—	1.75	7.25	5.50	2.50	2.—	3.—	3.25	0.75
—	—	—	—	0.75	—	0.25	—	—	1.—	0.25	0.50	—
70 100	178 000	10 000	91 100	218 400	66 890	86 400	73 200	117 000	129 400	54 500	43 200	111 900
9	—	—	—	3	19	22	5	7	—	—	4	19

## XI. Case Reports.

### *Key to Abbreviations in Case Reports.*

Asp.	= aspirated
Vac.	= vacuum
St.	= sternal
M.p.	= marrow particles
F.p.	= fat particles
A. pain	= aspiration pain
X cc vacuum	= the vacuum created when the plunger of the aspiration syringe is drawn back to the line of demarcation $\gamma$ cc.

Case 1. K. J. ♀ aged 39. Born 18—3—1898, Ullensaker. (Ref. nr. 7004/37—38).

Occupation: Wife of lumber-man.

In the late autumn of 1932 troubled by giddiness, headache, breathlessness, tingling in her hands, soreness of her tongue and loss of weight (about 10 kg.). Admitted 24—1—33 to the Med. Dept. A. of the Rikshospital where pernicious anaemia was diagnosed. Hb. 29%, erythr. 1.06 mill. Leucocytes 4700. Serum colour 9. Anacidity. Treatment with injections of hepsolin and discharged with erythr. 3.55 mill. Instructed to take 200 g. liver daily. Has subsequently been repeatedly admitted to the same Dept. for relapses, the last occasion being 7—2—1938.

She feels tired and relaxed, complains of headache, seems mentally dull with a touch of Mongolism about her. A hint of yellow in her skin, and her sclera are yellow. State of nutrition good. Blood press. 120/65. Pulse 92, regular. Resp. 20, not embarrassed. Temp. 38. Tongue moist, its margin smooth. Apex beat of the heart not visible. A systolic adventitious sound loudest over the apex. Liver dulness 6 c. to 2 finger-breadths below the costal arch. Margin of the liver palpable. Spleen palpable.

Reflexes:	R.	L.
Abdom. reflexes	+	+
Patel.       "	++	++
Achil.       "	++	++
Babinsk.   "	↓↓	↓↓
Gait unsteady. Romberg	÷	

Wassermann ÷. S. R. 156 mm. after 1 hr. (falling to 60 during stay in hospital). Serum colour 11. Ewald test meal: Congo ÷, Mc. Lean ÷, ac. 0/6. Urine: Schlesinger (1/10 dilut.) ÷. Hb. 40 % = 5.52 g. %. Hb. pr. erythr. 37.7 γγ. Erythr. 1.46 mill. Leucocytes 5000. Reticulocytes 6 %<sub>100</sub>. Blood smear: Praemyelocytes +, eos. 1.3 %, metamyelocytes 0.7 %, band forms. 3.2 %, polymorphs 51.9 %, monocytes 1.9 %, lymphocytes 40.3 %, plasma-Türk 0.7 %. Faint anisocytosis, macro-megalocytosis. A few microcytes, schizocytes and poikilocytes. Orthrochromasia. Polychromasia. Several basophil-punctuated erythrocytes. Cabot's rings and Jolly bodies. Over segmentation of the nuclei of the neutrophil leucocytes. Nucleated erythrocytes 3/500 L. Sternal puncture: Megalo-macro-blastosis.

A radiological examination of the stomach and duodenum negative.

Treatment: 12—2 20 cc. pernami nr. II.

28—2 20 cc. pernami nr. II.

Case 2. J. E. ♂ aged 63. (Ref. nr. 6787/35—36).

Occupation: Lumber-man.

Subject to dyspepsia for the last twenty years, particularly on heavy work. A sense of gastric oppression directly after meals, particularly

when they were heavy. Has felt more and more relaxed during the last two years and has been obliged gradually to reduce his work. Of late gradually becoming still more relaxed, he has been unable during the last three weeks to do any work, and he has spent most of his time in bed. Troubled of late by tinnitus and some soreness of his tongue. Has recently noticed he had become pale and yellow and had lost weight. The doctor he consulted secured his admission to the Med. Dept. A. of the Rikshospital on 20—2—36.

*Present condition:* Very pale, mucous membranes also pale. Face slightly but definitely yellow. Emaciated. Blood press. 100/55. Tongue smooth about its margins and sore. A slight systolic blowing murmur loudest over the apex beat. Pat. and Achil. reflexes absent. The patient states that he has often noticed that his hands and arms have become numb. Ewald test meal: ( $\frac{3}{4}$  hr.) Ac. 0/7, Congo  $\div$  McLean  $\div$ . Urine: Schlesinger (1/10 dilut.) + faint, otherwise normal. Wassermann  $\div$ . Serum colour 7. S. R. 36 mm. after 1 hr. Hb. 50 % = 6.9 g. %. Hb. pr. erythr. 45.1  $\gamma\gamma$ . Erythr. 1.53 mill. Reticulocytes 36 %<sub>100</sub>. Leucocytes 4600. Blood smear: Eos. 1 %, band forms 3.5 %, polymorphs 43.5 %, monocytes 0.5 %, lymphocytes 51.5 %. Polychromasia, marked anisocytosis, poikilocytosis, megalocytosis, schizocytosis and microcytosis. Anisochromia. Marked over-segmentation of the nuclei of the neutrophil cells. Macroblasts 1/200 L.

*Sternal puncture:* Megalo-macro-blastosis.

A radiological examination of the stomach and duodenum yielded normal findings.

*Treatment:* 24—2—36 11 cc. B-bu-perf-Eu-phase desalted four times. felling. 1 375 g. liver.

$\frac{2}{3}$ — $\frac{4}{3}$ —36 80 cc. pernami »Nyco».

*Case 3. A. R. ♀ aged 72. Born 13—7—1864, Aalesund. (Ref. nr. 914/36—37).*

*Occupation:* Housewife.

She has been troubled by diarrhoea all her life. Achylia demonstrated in 1926, HCL prescribed, and she felt better. Treated in the Med. Dept. A. of the Rikshospital oct. 1929 with the diagnosis of achylia gastrica and diarrhoea. Since May, 1935, pain and stiffness in both knees. Treated at the Sandefjord hydro. After April 1936 she began to feel listless and breathless, and she became more and more pale. Never any soreness of her tongue. In May, 1936, a doctor diagnosed pernicious anaemia. She felt somewhat better after liver injections, but was still tired, listless and out of breath. Defaecation and micturition have functioned normally of late. Admitted 6—7—36 to the Med. Dept. A. of the Rikshospital for pain in her knees and for insomnia.

*Present condition:* She looks and feels fit and does not give an impression of anaemia. Pulse 68 and regular. Blood press. 150/85. Temp. 37.6.

Case 1—2—3. Cell count and Hmglob. from ear and sternal blood.

	Retienocytes per mille		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear- blood	sternal- blood	ear- blood	sternal- blood	ear- blood	sternal- blood	ear- blood	sternal- blood
Case 1	10	7	36	48	1.39	1.80	3200	87800
Case 2	9	3.1	50	16	1.53	1.22	4600	51000
Case 3	2	1	110	64	4.58	2.41	4800	39000

Case: 1—2—3 Differential cell count of sternal blood.

Sternal puncture			Asp. under vac. (20 cc.) 0.3 cc. st. blood m.p. +++ f.p. + severe a pain		Asp. easy 0.35 cc. st. blood m.p. ++ +, f.p. ++ moderate a. pain
Erythropoiesis	Erythroblasts	per cent	27	45.8	15.4
	Leuco- blasts	"			
	cytes	"	73.--	54.2	84.6
	Promegalomacroblasts	"	1.--	—	—
	Megalobl. bas.	"	2.--	0.50	3.9
	eos.	"	2	0.50	—
	Macrobl. bas.	"	53.--	62.25	14.3
	eos.	"	26.--	—	14.3
	Normobl. bas.	"	—	—	15.6
	eos.	"	—	—	31.5
	Erythrobl. bas.	"	—	23.75	14.3
	eos.	"	14.--	12.75	3.9
Leucopoiesis	Erythrobl. bas.	"	2.--	0.25	1.3
	Division forms	eos.	—	—	1.3
	Megakaryocytes	"	—	—	0.50
	Mast cells immature	"	—	—	—
	mature	"	—	—	—
	Eos. myelocytes	"	1.75	0.25	1.--
	leucocytes band forms	"	2.75	1.25	1.--
	polymorphs	"	0.75	0.75	0.50
	Myeloblasts	"	3.--	1.--	1.75
	Praemyelocytes	"	4.--	5.50	3.75
	myelocytes	"	16.50	8.--	14.25
	Neutro- young forms	"	39.50	32.25	19.25
	philes band forms	"	2.25	23.--	16.25
	polymorphs	"	8.50	27.50	14.50
	Mono- blasts	"	} 0.25	—	0.75
	cytes	"			
	Lymphocytes	"	4.25	0.50	15.25
	Plasma & Türk cells	"	—	—	0.75
	Reticulo endothelial cells	"	1.75	—	1.75
	Smear cells	"	14.75	—	8.75



Heart and lungs normal, and liver and spleen not palpable. No rash or oedema. Urine normal. Ewald test meal: Congo  $\div$ , ac. 0/6. Hb. 110 % = 15.18 g. %. Hb. pr. erythr. 33.2  $\gamma\gamma$ . Erythr. 4.58 mill. Leucocytes: 4800. Reticulocytes 2. Serum colour 7. Blood smear: Bas. 1 %, eos. 3 %, band forms 3.5 %, polymorphs 55.5 %, lymphocytes 32.5 %, monocytes 4.5 %. Anisocytosis, microcytosis, schizocytosis, orthochromia, macrocytes, slight anisochromia, macrothrombocytes, no over-segmentation of the nuclei of the neutrophil leucocytes.

*Sternal puncture:* Megalo-macro-blastosis.

*Case 4. K. S. ♀ aged 56. Born 2—11—1880, Holt, Fet. (Ref. nr. 7857/36—37).*

*Occupation:* Wife of a master carpenter.

Albuminuria during two or three of her pregnancies, and on two occasions, when not pregnant, confined to bed for renal disease. She thinks that she has not suffered from it since the birth of her last child 14 years earlier. Six or seven years ago she began to be troubled by tiredness and lassitude and pain in the small of the back. A doctor diagnosed anaemia and prescribed 100 g. liver daily plus iron pills. In the autumn of 1936 she began again to suffer from tiredness, lassitude and loss of appetite. Attended the medical polyclinic of the Rikshospital 18—9—36 where anaemia was found. Hb. 87 %, erythr. 4.59 mill. Radiological examination of stomach and duodenum showed normal conditions. Iron pills and HCL were prescribed, and she has since taken them. But she did not improve, and in Nov. 1936 she began to suffer also from a sense of pricking in ankles and knees, and from defective hearing, tinnitus, giddiness and soreness of her tongue. Considerable loss of weight the past half year (exact loss not known). Anorexia, but she sleeps well, and there has been no disturbance of micturition. Constipated during the last month. Admitted to the Med. Dept. A. of the Rikshospital 22—3—37.

*Present condition:* The patient seems tired and apathetic. Some dysarthria and impaired hearing. Complexion pale-yellow. She is emaciated and complains of pain in her legs. Temp. 37.8, resp. 18 and not embarrassed. Pulse 100, regular. Blood press. 100/45. Tongue moist, smooth, atrophic papillae over the whole of it. No oedema, no rash. A hint of yellow in the sclerae. No absolute cardiac dulness. Systolic murmur loudest over the apex. Apex beat in the 5th intercostal space, 11 cm. from the middle line, outside the mid-clavicular line. Liver dulness 6 c. to 2 finger-breadths below the costal arch.

<i>Reflexes:</i>	R.	L.
Abdom. mid-reflexes	faint +	faint +
Abdom. upper reflexes	$\div$	$\div$
Abdom. lower reflexes	$\div$	$\div$
Patel.                »	$\div$	$\div$
Achil.                »	$\div$	$\div$
Plantar              »	faint	faint

Ewald test meal (after  $\frac{3}{4}$  hr.), ac. 0/16, McLean —, Congo —. Urine: trace of albumin, no pus, guaiac.  $\div$  Schlesinger (1/10 dilut.) +, Wassermann —. Serum colour 9. S. R. 42 (after 1 hr.), retics. 7200, Hb. 26 %—3.588 g. %. Hb. pr. erythr. 40.3  $\gamma\gamma$ . Erythr. 0.89 mill. Leucocytes 2600. Blood smear: eos. 0.5 %, myelocytes 1 %, metamyelocytes 4 %, band forms 6.5 %, polymorphs 35 %, lymphocytes 53 %. Marked anisocytosis, schizocytosis, microcytosis, macro-megalocytosis, oversegmentation of the nuclei of the neutrophil leucocytes. Orthochromia, poikilocytosis, polychromasia 1 %<sub>100</sub>. Macroblasts 1/200 L.

*Sternal puncture:* Megalo-macro-blastosis.

The patient's lassitude was steadily progressive, and she died 28—3—37.

Case 4. Differential leucocyte count of ear blood.

1937			22—3	23—3	24—3
Praemyelocytes per cent.....			—	0.5	—
Eos. " " .....			0.5	1.5	1.0
Neutrophils	Myelocytes	" " .....	1.0	1.0	0.5
	Young forms	" " .....	1.0	1.5	3.0
	Band forms	" " .....	6.5	6.5	23.5
	Polymorphs	" " .....	35.0	37.5	40.0
Monoocytes " " .....			—	1.0	—
Lymphocytes " " .....			53.0	50.5	32.0
Macrobl.	bas.	} in 200 leucocytes .....	—	1	1
	eos.		—	2	2
Erythrobl.	bas.	" .....	—	—	1
	eos.	" .....	1	—	1

Case 4. Cell count and Hmglob. from ear and sternal blood.

1937	Reticuloocytes per mille		Hmglob. pr. erythroc. $\gamma\gamma$		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear- blood	sternal -blood	ear- blood	sternal -blood	ear- blood	sternal -blood	ear- blood	sternal -blood	ear- blood	sternal -blood
22—3	71	90	40.3	40.7	26	23	0.89	0.78	2600	61800
23—3	72	114	40.9	44.8	24	24	0.81	0.78	2200	8400
24—3	70	88	40.5	39.2	22	23	0.75		2000	7800
"			27		37		0.89		2700	
25—3	23		29.4		35		1.64		1400	
26—3	21		35.3		33		1.29		2700	
27—3	22		39.8		32		1.11		2700	
"			34.3		50		2.01		1800	
28—3	27				64					

Case 4. Differential cell count of sternal blood.

1937		22—3	23—3	21—3	
Sternal puncture		Asp. easy 0.6 cc. st. blood m.p. + + + f.p. ÷ severe a. pain	Asp. easy 0.75 cc. st. blood. m.p.: traces f.p. ÷ moderat a. pain	Asp. easy 1.2 cc. st. blood m.p. ÷ f.p. ÷ no a. pain	
Erythropoiesis	Erythroblasts	per cent	35.5	29.6	26.5
	Leuco- blasts	»			
	cytes	»	64.5	70.4	73.5
	Promegalomaeroblasts	»	7.5	2.—	3.—
	Megalobl.	bas. »	5.5	7.—	3.—
		eos. »	—	—	—
	Macrobl.	bas. »	66.5	65.—	65.—
		eos. »	15.—	11.—	—
	Normobl.	bas. »	—	—	—
		eos. »	—	—	2.—
	Erythrobl.	bas. »	0.5	11.—	11.—
		eos. »	4.5	4.—	1.—
Erythrobl.	bas. »	0.5	—	—	
Division forms	eos. »	—	—	—	
Leucopoiesis	Megakaryocytes	»	0.25	0.50	—
	Mast cells	immature »	0.25	1.—	0.3
		mature »	—	—	0.3
	Eos- myelocytes	»	2.25	1.—	1.7
	leucocytes	band forms »	2.75	2.—	2.—
		polymorphs »	3.—	1.5	0.3
	Myeloblasts	»	6.75	4.—	1.7
	Praemyelocytes	»	7.50	1.5	6.3
	Neu- myelocytes	»	19.25	2.—	6.7
	trophiles	young forms »	26.75	13.—	13.7
		band forms »	4.75	9.5	13.—
		polymorphs »	3.25	16.—	19.7
	Mono- blasts	»	0.25	0.5	—
	cytes	»			
	Lymphocytes	»	11.25	38.—	21.—
	Plasma & Türk cells	»	0.25	—	—
Reticulo endothelial cells	»	0.25	—	—	
Smear cells	»	11.25	9.5	13.—	

A post-mortem examination showed medulla osseum rubra, enlargement of the spleen, haemorrhagic and gangrenous cystitis, suppurative pyelo-nephritis on the right side, chronic nephritis with scar formation. and bilateral pneumonia.

*Treatment:* On 25—3—1937 transf. sang. 500 cc. and on 27—3 1000 cc.  
 22—3 2 cc. Mrk. (X) E = 1 mg. t.s.  
 24—3 10 cc. Mrk. (X) E = 5 mg. t.s.

*Case 5. J. M. ♂ aged 46. (Ref. nr. 9281/35—36).*

*Occupation:* Carpenter.

Healthy till 1927 when he was troubled for 4 to 5 months by dyspepsia manifesting itself as a dragging pain in the epigastrium whence it radiated to the small of the back. It was apt to occur before meals and to be relieved on eating. Troubled also by diarrhoea. A doctor found that he lacked HCL, but though this was prescribed, he continued to suffer from diarrhoea. Early in August, 1935, progressive lassitude. He had to give up work 1—4—1936, at which date his old dyspepsia recurred. No loss of weight. Admitted to the Med. Dept. A. of the Rikshospital 11—5—1936.

Present condition: Rather pale and slightly jaundiced. Tongue much furrowed ÷ not smooth. (The patient says, however, that his tongue has been sore for about one year). Pulse 60, regular. Temp. 37.6. Resp. 20, not embarrassed. Blood press. 150/90. Heart normal. Urine: Schlesinger +. Ewald test meal: (¾ hr.) ac. 0/4, McLean ÷, Congo ÷. Wassermann ÷.

Case 5. Differential leucocyte cell count of ear blood.

1936		14—5	15—5	16—5	17—5
Bas.	per cent .....	—	0.5	—	—
Eos.	" " .....	1.5	2.5	2.5	1.5
Neutro- phils	band forms " " .....	2.0	1.0	2.5	1.5
	(polymorphs " " .....	53.0	57.0	61.0	56.0
Monoocytes	" " .....	1.0	2.0	0.5	3.5
Lymphocytes	" " .....	42.5	37.0	33.5	37.5
Erythrobl.	in 200 leucocytes	—	—	—	—

Case 5. Cell count and Hmglob. from ear and sternal blood.

1936	Reticuloocytes per mille		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
14—5	9	10	61	49	2.77	1.60	5900	7070
15—5	9	17	58.5	59	2.01	1.97	5600	64600
16—5	8	14	41.5	59.5	2.00	1.65	5800	41300
17—5	12	15	61.—	51.—	1.90	1.90	4800	57800
26—5	22		59.—		2.20		6000	
7—6	109							
15—6	6		90.—		3.48		6000	

Case 5. Differential cell count of sternal blood.

1936			14—5	15—5	16—5	17—5
Sternal puncture			Asp. easy 0.35 cc. st. blood m.p.: 0 f.p.: + + + + + no asp. pain	Asp. easy 0.55 cc. st. blood m. p.: + + f.p.: 0 moderate a. pain	Asp. easy 0.4 cc. st. blood m.p.: + f.p.: 0 moderate a. pain	Asp. easy 0.85 cc. st. blood m.p.: + f.p.: 0 moderate a. pain
Erythropoiesis	Erythroblasts	per cent	—	23.—	31.—	27.8
	Leuco- blasts	"	—	77.—	69.—	72.2
	cytes	"	—	—	—	—
	Promegalomacroblasts	"	—	—	—	—
	Megalobl.	bas.	+ —	13.0	4.5	8.6
		eos.	—	—	2.6	0.7
	Macrobl.	bas.	—	46.1	48.5	39.0
		eos.	—	5.2	12.2	1.4
	Normobl.	bas.	—	3.5	2.6	12.2
		eos.	—	—	5.2	0.7
	Erythrobl.	bas.	+ (1)	13.9	13.5	15.8
		eos.	—	15.7	7.7	17.3
	Erythrobl.	bas.	—	2.6	3.2	4.3
	Division forms	eos.	—	—	—	—
Leucopoiesis	Megacaryocytes	"	—	—	—	—
	Mast cells	immature	—	—	0.50	0.25
		mature	0.5	—	—	—
	Eos.	myelocytes	—	1.25	1.25	1.25
	leucocytes	band forms	—	0.75	1.00	1.25
		polymorphs	0.5	1.00	2.25	2.75
	Myeloblasts	"	—	2.00	1.50	1.25
	Praemyelocytes	"	—	5.00	3.00	7.75
	Neutro-	myelocytes	—	10.25	10.25	10.50
	philes	young forms	1.0	16.50	14.50	22.—
		band forms	0.5	12.50	14.75	17.75
		polymorphs	66.5	25.50	19.25	16.50
	Mono-	blasts	}	1.00	—	1.50
		cytes		—	—	—
	Lymphocytes	"	27.0	14.50	18.75	13.—
	Plasma & Türk cells	"	—	0.25	1.00	0.50
	Reticulo endothelial cells	"	—	—	0.25	—
	Smear cells	"	4 —	9.50	11.75	3.75

Serum colour 10. Hb. 57 % = 7.87 g. %, erythr. 2.31 mill. Hb. pr. erythr. 34.1  $\gamma\gamma$ . L. 5300. Blood smear: eos. 1 %, band forms 1.5 %, polymorphs 30.5 %, monocytes 0.5 %, lymphocytes 66.5 %. Marked anisocytosis, schizocytosis, microcytosis, macrocytosis, megalocytosis, anisochromia, polychromasia, basophil punctuation, erythroblasts 1/200 L., and over-segmentation of the nuclei of the neutrophil cells.

*Sternal puncture:* Macro-megaloblastosis.

A radiological examination of stomach and bulb. duod. showed normal conditions.

*Treatment:* 14—5—36 4. cc. Bentz filtr. XX=200 g. liver.

26—5—36 4.5 cc. Bentz filtr. = 250 g. liver.

2—3/6—36 40 cc. Pernami.

*Case 6. J. B. ♂ aged 65. (Ref. nr. 9557/35—36).*

*Occupation:* Retired farmer.

Since 1916 troubled by rheumatism for a couple of years. Diabetes diagnosed in 1934. Put on a diet and felt well till about a week ago when he began to experience lassitude and tiredness as well as breathlessness on walking upstairs. He continued to work till 14—5 when he had to give it up on account of epistaxis about 1/4 liter. Admitted to the Med. Dept. A. of the Rikshospital 20—5—1936.

*Present condition:* The patient is very pale and thin. Tongue moist and clean. Pulse 80 and regular. Temp. 37.6. Resp. 20 and not embarrassed. Blood press. 120/70. Apex beat 1 cm. outside the nipple line. Liver and spleen not palpable. Diffuse swelling of both wrists which are completely ankylosed. The slightest attempt to induce movement provokes con-

Case 6. Differential leucocyte count of ear blood.

1936		21—5	25—5	26—5	27—5	28—5	23—6
Praemyelocytes	per cent	0.5	—	—	—	—	—
Bas.	" "	0.5	0.5	—	—	—	—
Eos.	" "	+	1.5	1	1.0	—	1.0
Neutrophils	myelocytes	—	—	1	—	—	—
	young forms	—	0.5	1	1.5	—	2.0
	band forms	4.0	15.5	19	18.0	11	27.5
	polymorphs	29.0	28.0	30	19.5	15	29.5
Monocytes	" "	1.5	—	1	0.5	—	10.5
Lymphocytes	" "	64.5	54.0	47	59.5	74	30.0
Macrobl. bas.	in 200 leu-	—	—	—	—	—	—
eos.	leucocytes.	—	—	2	—	—	—
Erythrobl. bas.	"	1	—	—	—	—	—
eos.	"	—	—	2	—	—	—

Case 6. Cell count and Hmglob. from ear and sternal blood.

1936	Reticulocytes per mill.		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
21—5			35.—		1.67		2900	
23—5	14		37.—		1.62		2400	
25—5	8	15	35.—	33.5	1.56	1.59	1800	41700
26—5	10	18	38.5	43.0	1.34	1.45	1700	19100
27—5	7	9	35.—	35.—	1.52	1.28	1800	28200
28—5	6	9	35.—	32.—	1.32	1.05	1200	49000
29—5	7							
30—5	6		25.—		1.18		2800	
4—6	181		36.—		1.89		2400	
20—6	9		66.—		3.04		2200	

siderable pain. Reflexes normal. Urine: Benedict ++, Gerhard ÷ Rothera ÷, sp. gr. 1030. Wassermann ÷. Serum colour 9. S.R. 56 mm. after 1 hr. Hb. 35 % = 4.37 g. %. Hb. pr. erythr. 28.5 γγ. Erythr. 1.67 mill. L. 2900. Reticulocytes 9‰. Blood smear: bas. 0.5 %, eos +, praemyelocytes 0.5 %, band forms 4 %, polymorphs 29 %, lymphocytes 64.5 %, monocytes 1.5 %. Megalo-macrocytosis, anisocytosis, microcytosis, schizocytosis. Erythroblasts 1/200 L. Polychromasia and anisochromia. No over-segmentation of the nuclei of the leucocytes.

*Sternal puncture:* Macro-megaloblastosis.

No growth on blood culture.

*Treatment:* 25—5—36 3 cc. Bentz filtr. = 150 g. liver.

29 and 30—5 40 cc. Pernami.

*Case 7. ♀ B. II. aged 26. Born 28—12—1908, Soknedal (Ref. nr. 6463/36—37).*

*Occupation:* Wife of milkman.

Became pregnant in July 1936, and during the first half of her pregnancy she suffered somewhat from nausea and, on rare occasions, vomited. At the end of Nov. 1936 (about 2 ½ months earlier) she began to suffer from lassitude, losing her appetite and becoming definitely pale. She lost weight and, finding it difficult to work in the cow byre, she ceased work 11—12—36, when she suffered from attacks of shivering and sweating, frontal headache, epistaxis and pain in her knees, calves, forearms and between her shoulder-blades. She had also a violent cough with profuse expectoration which was often brown or blood-stained. This expectoration was usually a sequel to her nose-bleeding from which she suffered more or less every day. A doctor summoned on 12—12—36 diagnosed influenza.

Case 6. Differential cell count of sternal blood.

1936			25—5	26—5	27—5	28—5
Sternal puncture			Asp. easy 0.85 cc. st. blood, m.p. + f.p. 0	Asp. under vac. 0.9 cc. st. blood m.p. traces f.p. traces	Asp. under vac. 0.7 cc. st. blood m.p. + f.p. 0	Asp. easy 1.3 cc. st. blood m.p. + f.p. 0
Erythropoiesis	Erythroblasts	per cent	41.6	52.6	45.4	45.8
	Leuco- blasts	"				
	cytes	"	58.4	47.4	54.6	54.2
	Promegalomacroblasts	"	1. —	0.8	—	1.3
	Megalobl. bas.	"	8.2	5.3	2.2	9.5
	eos.	"	—	2.7	—	1.3
	Macrobl. bas.	"	38. —	38.7	56.3	46.7
	eos.	"	3.8	5.3	1.8	11.7
	Normobl. bas.	"	—	—	1.8	0.4
	eos.	"	—	—	—	—
	Erythrahl. bas.	"	38.4	21.7	19.4	14.3
	eos.	"	10.1	25.5	16.3	12.6
	Erythrobl. bas.	"	0.5	—	2.2	2.2
	division forms eos.	"	—	—	—	—
Leucopoiesis	Megacaryocytes	"	—	—	—	—
	Mast cells immature	"	0.25	—	—	0.25
	mature	"	—	—	—	—
	Eos. myelocytes	"	1.50	2.50	1.50	1.75
	leucocytes band forms	"	2.25	0.50	0.50	1.00
	polymorphs	"	0.75	0.25	1.00	1.00
	Myeloblasts	"	1.00	1.50	1.00	1.25
	Praemyelocytes	"	2.25	5.25	3.25	4.00
	myelocytes	"	18.25	13.50	10.50	16.25
	Nentro- young forms	"	24.00	22.00	14.25	21.50
	philes band forms	"	18.00	11.25	16.75	8.40
	polymorphs	"	8.00	7.75	9.25	5.25
	Mono- blasts	"				
	cytes	"	1.75	1.75	0.75	1.00
Lymphocytes			13. —	16.00	28. —	23.25
Plasma & Türk cells			—	0.25	—	—
Reticulo endothelial cells			0.75	0.50	0.25	0.75
Sinus cells			8.25	13.50	13. —	14.25



She got no better, and on 30—12—36 she was sent to a diagnosis station in the County of Aker where she was found to be Pirquet-positive, S.R. 70. The X-rays showed enlarged hilus shadows on both sides and, on the right side, a few small patches in the lung itself. These were not interpreted as indicating active tuberculosis. A renewed radiological examination on 3—2—37 showed recession of the shadows in the right hilus. She stated that for several years she had suffered from time to time from tingling in her fingers and toes, and that the tip of her tongue had been sore latterly. On 6—2—37 she was admitted to the Med. Dept. A. of the Rikshospital.

Present condition: She is very pale. Resp. 28, audible, temp. 38.2, tongue moist and clean, its surface rather flat. Blood press. 95/40. No oedema nor rash. Nothing abnormal about her throat, neck or lungs. Apex beat in the fifth intercostal space 10 cm. from the middleline, dullness fourth left rib to margin of sternum. A faint systolic murmur loudest at insertion of third rib. Liver and spleen not palpable. Abdomen large, fundus uteri 1/3 supra-umbilic. Foetal heart sounds audible below the umbilicus, approximately in the middle line. Ewald test meal: Congo ÷, McLean ÷, ac. 0/6 after 3/4 hr. Basal metabolism 123 %. Wassermann ÷.

Case 7. Differential leucocyte count of earblood.

1937		8—2	14—2	16—2
Eos.	per cent .....	1	2.0	0.5
Neutrophils {	myelocytes » » .....	1	1.5	2.0
	young forms » » .....	1	2.0	3.0
	band forms » » .....	4	8.5	11.0
	polymorphs » » .....	60	67.0	57.0
Monocytes	» » .....	3	1.0	0.5
Lymphocytes	» » .....	30	18.0	26.0
Megalobl.	bas. in 20 leucocytes .....	1	—	—
	eos. ....	—	—	—
Macrobl.	bas. » .....	1	—	—
	eos. » .....	—	—	1

Case 7. Cell count and Hmglob. from ear and sternal blood.

1937	Reticulocytes per mille		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
6—2			34		1.72		2300	
13—2	6	6	37	37.5	1.68	1.53	2700	68900
14—2	1							
16—2	5	10	40	39.—	1.95	1.64	3600	20500

Case 7. Differential cell count of sternal blood.

1937			13—2	16—2
Sternal puncture			Asp. easy 0.4 cc. st. blood. m.p. + + + + f.p. 0 severe a. pain	Asp. under vac. (10 cc.) 0.9 cc. st. blood. m.p. traces, f.p. 0 severe a. pain
Erythropoiesis	Erythroblasts	per cent	37.5	30.9
	Lenco- blasts	%		
	cytes	%	62.5	69.1
	Promegalomacroblasts	%	11.5	7.—
	Megalobl. bas.	%	10.5	8.—
	eos.	%	—	—
	Macrobl. bas.	%	78.—	49.—
	eos.	%	—	20.—
	Normobl. bas.	%	—	—
	eos.	%	—	—
	Erythrobl. bas.	%	—	2.—
	eos.	%	—	1.—
	Erythrobl. bas.	%	—	3.—
	division forms	eos. %	—	—
Leucopoiesis	Megacaryocytes	%	—	0.25
	Mast cells immature	%	—	0.25
	mature	%	—	0.25
	Eos. myelocytes	%	0.50	0.75
	leucocytes band forms	%	2.75	2.75
	cytes polymorphs	%	1.00	3.—
	Myeloblasts	%	1.50	4.—
	Praemyelocytes	%	6.50	4.25
	Neutro- myelocytes	%	21.50	5.75
	philles young forms	%	34.75	21.25
	band forms	%	10.75	13.75
	polymorphs	%	8.25	20.75
	Mono- blasts	%	0.75	0.25
	cytes	%		
	Lymphocytes	%	5.—	17.50
	Plasma & Türk cells	%	—	—
	Reticulo endothelial cells	%	—	—
	Smear cells	%	6.75	5.25

S.R. 60 mm. after 1 hr. Urine: no protein, no pus, guaiac  $\div$ , Benedict  $\div$ , Schlesinger (1/10 dilut.) ++. Serum colour 4.5. Hb. 34 % = 4.692 g. %. Hb. pr. erythr. 27.3  $\gamma\gamma$ . Erythr. 1.72 mill. Leucocytes 2300. Blood smear: eos. 1 %, myelocytes 1 %, m. myelocytes 1 %, band forms 4 %, polymorphs 60 %, monocytes 3 %, lymphocytes 30 %. Great anisocytosis, schizocyto-

sis, microcytosis, poikilocytosis, macrocytosis, and megalocytosis. One megaloblast and one macroblast. Carbot's rings. Polychromasia 2%. Some of the neutrophil leucocytes show over-segmentation.

*Sternal puncture:* Megaloblastosis.

On 12—2—37 she developed a sore throat with fever which, in the course of a couple of days, rose to 40 on account of a progressive phlegmonous inflammation which extended from her neck down to the middle of her thorax. Death occurred on 18—2—37 after extensive incisions made in vain at the Surg. Dept. B. of the Rikshospital.

*Treatment:* 10—2—37 500 cc. and 17—2—37 1000 cc. defibrinated blood.

*Case 8. J. B. ♀ aged 69. Born 9—11—1867, Ullensaker. (Ref. nr. 9337/36—37).*

*Occupation:* Widow.

Was troubled in 1929 by dyspepsia, and a doctor prescribed a diet, but she became steadily worse, felt tired and listless, ate very little and became pale and thin. Her tongue was so sore that she could eat only tepid food. Her fingers and toes were liable to become cold and white, and they were very subject to a sense of pricking and stabbing. In June 1930 she attended the Medical Polyclinic of the Rikshospital, was given liver treatment, and became perfectly well. At first she ate 200 g. of liver daily, but after some time she discontinued it altogether. She was well and at work till just before Christmas 1930 when she lost weight, vomited all her food, and suffered from her earlier symptoms. Between 29—7—1931 and 17—9—1931 she was treated for pernicious anaemia and thyreotoxicosis at the Med. Dept. B. of the Rikshospital. On admission: Erythr. 1.47 mill. L. 1900. Serum colour 14. Retics.  $\frac{1}{2}$  %. Was treated with campolon and liver. On discharge: Erythr. 3.98 mill. L. 4800.

Thereafter for a year she ate 250 g. fried liver daily. But gradually she came to omit this treatment for several days, and the quantity of liver consumed diminished when her appetite dwindled. In February, 1935, a doctor gave her injections, and she became well. At first she did not eat any liver, but since May, 1936 she ate about 50 g. weekly. Since then her troubles have gradually increased, and she was transferred from the Polyclinic on 8—5—1937 to the Med. Dept. A. of the Rikshospital.

*Present condition:* She is very pale and thin, and her conjunctivae are also pale. Tongue red, moist, clean. Pulse 76, regular. Resp. 16, unembarrassed. Temp. 38.2. Blood press. 165/80. Pupils equal in size, circular, reacting to light and accommodation. A goitre, of the size of a goose's egg. Apex beat visible in the nipple line in the fifth intercostal space. Cardiac diam. 15 cm. Blowing systolic murmur over the apex. Liver palpable 3 finger-breadths below costal arch. Spleen firm and palpable 2 finger-breadths below the costal arch. Reflexes normal. Urine: Schlesinger (1/10 dilut.) ++ Wassermann ÷. Serum colour 11. S.R. 39 mm. after 1 hr. Ewald test meal

(after 3/4 hr.) Congo  $\div$ , McLean  $\div$ . Standard basal metabolism 157 %. Hb. 61 % = 8.418 g. %. Hb. pr. erythr. 39.5  $\gamma\gamma$ . Erythr. 2.13 mill. Retics. 6 %<sub>00</sub>. Leucocytes 1900. Blood smear: eos. 2.6 %, band forms 8.6 %, polymorphs 19.8 %, monocytes 4.3 %, lymphocytes 64.7 %. Anisocytosis, schizocytosis, poikilocytosis, macro-megalocytosis, basophil punctuation, polychromasia 1.5 %<sub>00</sub>. Over-segmentation of the nuclei of the neutrophil leucocytes.

Case 8. Differential leucocyte count of ear blood.

1937			8—5	28—5	21—6
Bas.	per cent	.....	—	0.5	—
Eos.	»	»	2.6	0.5	2.0
Neutrophils {	myelocytes	»	—	0.5	—
	young forms	»	8.6	0.5	1.0
	band forms	»	19.8	2.5	10.5
	polymorphs	»	4.3	13.0	56.5
Monocytes	»	»	4.0	13.0	5.—
Lymphocytes	»	»	60.7	69.5	25.0
Normobl. bas. in 200 leucocytes			—	5	—
eos.			—	6	—
Erythrobl. bas. »			—	—	—
eos. »			—	4	—

Case 8. Cell count and Hmglb. from ear and sternal blood.

1937	Reticulocyt. per mille		Hmglb. pr. erythroc. $\gamma\gamma$		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
8—5	6		39.5		61		2.13		1900	
12—5	8		34.5		67		2.68		1600	
13—5	9	9		39		56.—		2.66		50000
18—5	11		40.6		53		1.80		1700	
19—5	7									
20—5	12									
21—5	6		37.—		52		1.94		1300	
22—5	6									
23—5	10									
24—5	9		35.9		50		1.92		2000	
27—5	60		37.6		49		1.80		2800	
28—5	272									
7—6	21		38.2		67		3.00		2700	
21—6	9		28.6		85		4.10		2800	

Case 8. Differential cell count of sternal blood.

19			13.5
Sternal puncture			Asp. easy 0.95 cc. st. blood m.p.: ++ f.p.: traces
Erythropoiesis	Erythroblasts	per cent	46.9
	Leuco- blasts	»	
	cytes	»	53.1
	Promegalomacroblasts	»	8.5
	Megalobl.	bas. »	7.5
		eos. »	—
	Macrobl.	bas. »	65.—
		eos. »	8.5
	Normobl.	bas. »	1.—
		eos. »	1.—
	Erythrobl.	bas. »	1.—
		eos. »	6.5
	Erythrobl.	bas. »	1.—
	division forms	eos. »	—
Leucopoiesis	Megacaryocytes	»	+
	Mast cells	immature »	0.25
		mature »	—
	Eos.	myelocytes »	1.50
	leucocytes	band forms »	0.50
		polymorphs »	1.25
	Myeloblasts	»	4.—
	Praemyelocytes	»	5.75
	Neutro-	myelocytes »	19.25
	philes	young forms »	20.75
		band forms »	8.50
		polymorphs »	4.25
	Mono-	blasts »	0.75
		cytes »	
	Lymphocytes	»	13.75
	Plasma & Türk cells	»	1.25
	Reticulo endothelial cells	»	1.25
	Smear cells	»	17.—

*Sternal puncture:* Macro-megalo-blastosis.

Normal findings on radiological examination of the stomach and duodenum.

*Treatment:* 24—5—37 20 cc. Pernami »Nyco».

25—5—37 40 cc. Pernami »Nyco».

Case 9. I.U. ♀ aged 61. Born 9—5—1875, Skedsmo. (Ref. nr. 3301/36—37).

Occupation: Wife of moulder.

In the autumn of 1914 pneumonia followed by empyema for which an operation was performed. Otherwise well till Nov.—Dec. 1935 when she began to suffer from soreness of the mouth, the tongue in particular. Since then this condition has returned in bouts. Since July 1936 has felt tired, and the iron prescribed by a doctor had no effect. Hence his advice that she should attend the Medical Polyclinic whence she was admitted to the Med. Dept. A. of the Rikshospital. Since midsummer troubled by numbness and pricking and tingling in her fingers, so marked that it was difficult to hold her needle when sewing. During the last three months some headache and giddiness. Appetite poor.

Case 9. Differential leucocyte count of ear blood.

1936			22—10	24—10	5—11	7—11	9—11
Bas.	per cent	.....	1	—	1.0	0.5	2.0
Eos.	»	»	4	2	2.5	1.5	4.5
Neutrophils	young forms	»	—	—	0.5	2.5	2.5
	band forms	»	8	10	10.5	6.0	9.5
	polymorphs	»	22	25	40.0	14.5	35.0
Monocytes	»	»	7	9	8.5	4.5	6.5
Lymphocytes	»	»	58	54	36.0	70.5	40.0
Plasma Türk	»	»	—	—	1.0	—	—

Case 9. Cell count and Hmglob. from ear and sternal blood.

1936	Reticulocytes per mille		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
5—11	14	9	74	64.—	3.47	3.48	9200	57800
6—11	5							
7—11	3	5	74	73.—	3.38	3.077	5400	24200
8—11	14							
9—11	16	25	78	80.—	3.84	2.875	6100	30500
10—11	13							
11—11	14	13	80	66.5	3.76	2.70	5600	32200
13—11	3		80		4.02		7600	
16—11	9		80		4.17		7200	
18—11	6		83		4.26		7500	
21—11			84		4.11		8700	

Case 9. Differential cell count of sternal blood.

1936			5-11	7-11	9-11	11-11
Sternal puncture			Asp. under vac. (10 cc.) 0.1 cc. st. blood. m.p.: ++ f.p.: ++ moderate a. pain	Asp. easy 0.6 cc. st. blood m.p.: ++ f.p.: ++ moderate a. pain	Asp. easy 0.3 cc. st. blood m.p.: ++ f.p.: ++ moderate a. pain	Asp. easy 0.3 cc. st. blood m.p.: ++ f.p.: ++ moderate a. pain
Erythropoiesis	Erythroblasts	per cent	16.8	15.2	21.2	22.—
	Leuco- blasts	"	83.2	84.8	78.8	78.—
	cytes	"	—	—	—	—
	Promegalomacroblasts	"	—	—	—	—
	Megalobl. bas.	"	—	—	—	—
	cos.	"	—	—	—	—
	Macrobl. bas.	"	20.—	13.—	11.—	1.—
	cos.	"	3.—	7.—	7.—	—
	Normobl. bas.	"	56.—	60.—	66.—	84.—
	cos.	"	20.—	20.—	15.—	15.—
	Erythrobl. bas.	"	1.—	—	—	—
	cos.	"	—	—	—	—
	Erythrobl. bas.	"	—	—	1.—	—
	division forms cos.	"	—	—	—	—
Leucopoiesis	Megacaryocytes	"	—	—	—	—
	Mast cells immature	"	0.25	0.25	0.50	0.25
	mature	"	—	0.50	0.75	0.25
	Eos. myelocytes	"	0.75	1.50	1.—	0.75
	leucocytes band forms	"	1.75	2.00	2.—	1.50
	polymorphs	"	0.50	2.50	1.—	1.75
	Myeloblasts	"	0.25	1.50	1.50	0.25
	Praemyelocytes	"	4.—	3.50	4.75	3.25
	Neutro- myelocytes	"	22.25	16.—	16.—	23.25
	philes young forms	"	40.25	27.75	33.75	31.50
	band forms	"	14.50	11.75	9.50	17.25
	polymorphs	"	6.50	14.25	14.50	9.25
	Mono- blasts	"	0.50	4.25	3.25	2.25
	cytes	"	—	—	—	—
	Lymphocytes	"	0.75	7.75	3.—	4.50
	Plasma & Türk cells	"	0.50	0.75	0.50	0.50
	Reticulo endothelial cells	"	0.25	0.25	0.25	—
	Smear cells	"	7.—	3.25	7.75	3.50
	Unclassified cells	"	—	0.25	—	—

Present condition: 21—10—1936. General state of nutrition normal, skin yellow. Tongue moist, red, smooth, clean. Pulse 56, regular. Resp. 16, unembarrassed. Temp. 37.1. Blood press. 115/60. Cardiac dullness fourth rib to left of sternum. Apex beat not palpable. Total cardiac diam. 11.5 cm. First heart sound muffled. Accentuation of the second sound. Sharp systolic blowing murmur loudest over the apex. Hepatic dullness from 7th. rib to 2 fingerbreadths below costal margin, palpable. Reflexes normal. Urine: Schlesinger  $\div$  (1/10 dilut.). Ewald test meal: (3/4 hr.) ac. 0/5, Congo  $\div$ , McLean  $\div$ . Wassermann  $\div$ . Serum colour 4. S.R. 50 mm. after 1 hr. Hb. 73 % = 10.074 g. %. Hb. pr. erythr. 30.7 % Erythr. 3.28 mill. l. 4200. Retic. 5 ‰. Blood smear: eos. 4 %, bas. 1 %, band forms 8 %, polymorphs 22 %, monocytes 7 %, lymphocytes 58 %. Anisocytosis, poikilochromasia, orthochromia, slight schizocytosis and microcytosis. Carbot's rings. Marked basophil punctuation. A macrocyte here and there.

Sternal puncture: macro-normo-blastosis.

Normal conditions found on radiological examination of the stomach and bulb. duod. Heart seen to be slightly enlarged (aorta configuration).

Treatment: 5—11—36, 10 cc. Mrk. E<sup>b</sup> Ph. (3—11—36) = 500 g. liver.  
11—11—36, 10 cc. Mrk. E<sup>b</sup> Ph. (3—11—36) = 500 g. liver.

*Case 10. H. N. O. ♂ aged 69. Born 19—1—1865, Hallingdal (Ref. nr. 10670/36—37).*

*Occupation:* Farmer.

Well till 1931 when he began to feel that food was retained in his stomach. Some time later he consulted a doctor for increasing lassitude. This doctor found Hb. 42 %, and prescribed iron. The patient retired to a mountain dairy and felt well during the autumn. Latterly he has suffered more from lassitude, and has also noticed increased dyspnoea when at work. A year ago he consulted a doctor who found heart disease and prescribed medicine accordingly. Of late years friends have remarked that he looked rather pale, but during the last half year his pallor has become much more severe and his strength has failed considerably. Since February he has noticed soreness of his tongue, and latterly he has felt chilled. No paraesthesias of hands or feet, but his lower limbs have become weaker, and his urine darker. He sleeps and eats well, but fancies he has lost some weight. He was admitted to the Med. Dept. A. of the Rikshospitalet 17—6—1937.

*Present condition:* He is thin, and his skin is of a pale-yellow, straw-like colour. Mucous membranes pale and sclerae slightly jaundiced. Weight 54.5 kg. Weight when well was 75 kg. Increased irritability of the muscles. Tongue smooth. Pupils equal, reacting to light and accommodation. Fundus oculi: media not quite clear, nothing morbid visible. Pulse 80, regular except for a few extrasystoles. Temp. 37.1. Blood press. 140/95. Cardiac diam. 12 cm., its left limit 8 cm. from the middle line. A very faint



systolic murmur over the apex. Liver and spleen not palpable. Reflexes normal. Urine: dark brown, Schlesinger (1/10 dilut.) +, Wassermann ÷. Serum colour 12. S.R. 50 after 1 hr. Ewald test meal: (after 3/4 hr.) Congo ÷, McLean ÷, ac, 0/3. Hb. 37 % = 5.106 g. %. Hb. pr. erythr. 45.6 γγ. Erythr. 1.12 mill. Retics. 32 %. Leucocytes 2700. Blood smear: praemyelocytes 0.5 %, eos. 1 %, myelocytes 0.5 %, band forms 5.5 %, polymorphs 28.5 %, monocytes 3.5 %, lymphocytes 60 %. Marked anisocytosis, macro-megalo-cytosis, microcytosis, schizocytosis, poikilocytosis, orthochromia, basophil punctuation, polychromasia. Carbot's rings. Over-segmentation of the nuclei of the neutrophil leucocytes. Erythroblasts 4/200 L.

Sternal puncture: macro-megalo-blastosis.

Treatment: 18—6—1937 4 cc. Examin »Nycos« = 200 g. liver.

Case 10. Differential leucocyte count of ear blood.

1937		18—6	21—6	22—6	24—6
Praemyelocytes	per cent	0.5	—	0.5	—
Eos	" "	1.—	3.5	1.—	—
Neutrophils	myelocytes	0.5	0.5	1.—	—
	young forms	—	—	—	1.—
	band forms	5.5	3.—	1.5	4.—
	polymorphs	28.5	22.5	28.—	46.—
Monocytes	" "	3.5	3.—	10.5	6.5
Lymphocytes	" "	60.5	67.5	57.5	42.5
Normobl.	bas. in 200 leucocytes	—	—	—	2.—
	eos.	—	1.—	—	—
Macrobl.	bas.	1.—	—	—	—
Erythrobl.	eos.	3.—	1.—	2.—	—

Case 10. Cell count and Hmglb. from ear and sternal blood.

1937	Retienleucyt. per mille		Hmglb. pr. erythrocyt. γγ		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
18—6	32	41	45.6	46.9	37	34.—	1.12	1.00	2700	71000
19—6	40	46	38.6	47.6	33	35.—	1.18	1.015	1800	65000
20—6	41									
21—6	37	52	41.7	42.—	36	35.5	1.19	1.165	2600	111900
22—6	110	157	46.6	50.6	37	37.—	1.11	1.02	2800	63000
23—6	136									
24—6	114		40.9		40		1.35		3100	

Case 10. Differential cell count of sternal blood.

19			18—6	19—6	21—6	22—6
Sternal puncture			Asp. easy 0.8 cc. st. blood m.p.: + + + + f.p.: 0 severe a. pain	Asp. under vac. 0.8 cc. st. blood. m.p.: + + + f.p. 0 severe a. pain	Asp. under vac. (20 cc.) 0.9 cc. st. blood m.p.: + + + + f.p.: 0 severe a. pain	Asp. easy 0.5 cc. st. blood m.p.: + + + + f.p.: 0 severe asp. pain
Erythropoiesis	Erythroblasts	per cent	47.6	53.2	55.—	45.7
	Leuco- blasts	»				
	cytes	»	52.4	46.7	45.—	54.3
	Promegalomacroblasts	»	3.—	7.5	2.7	4.5
	Megalobl. bas.	»	3.5	5.5	1.7	+
	eos.	»	—	—	—	—
	Macrobl. bas.	»	31.—	44.—	47.3	33.5
	eos.	»	24.5	19.5	25.3	6.—
	Normobl. bas.	»	—	1.—	9.3	25.5
	eos.	»	—	—	1.7	16.—
	Erythrobl. bas.	»	2.—	0.5	2.—	4.5
	eos.	»	35.—	18.—	10.—	8.5
	Erythrobl. bas.	»	—	3.5	—	1.5
	division forms eos.	»	1.—	0.5	—	—
Leupopoiesis	Megacaryocytes	»	—	—	—	—
	Mast cells immature	»	0.50	0.25	0.50	0.25
	mature	»	0.25	—	—	—
	Eos. myelocytes	»	0.50	2.—	1.—	1.25
	leucocytes band forms	»	0.50	2.—	1.—	3.—
	polymorphs	»	—	1.50	1.—	1.50
	myeloblasts	»	1.25	2.75	0.75	1.25
	Praemyelocytes	»	6.25	6.25	3.75	3.—
	myelocytes	»	12.—	11.75	10.25	15.—
	Neutro- young forms	»	32.—	34.50	32.50	26.—
	philes band forms	»	5.50	7.50	5.25	8.25
	polymorphs	»	4.75	6.—	5.75	9.75
	Mono- blasts	»	}	1.50	0.25	1.—
	cytes	»				
	Lymphocytes	»	14.50	5.50	6.50	4.—
	Plasma & Türk cells	»	— .50	0.75	—	1.25
	Reticulo endothelial cells	»	—	0.25	1.25	1.25
	Smear cells	»	21.50	17.50	30.25	23.25



Case 11. W. H. ♂ aged 48. Born 27—10—1887, Löken, Höland. (Ref. nr. 10419/35—36).

Occupation: Saw-mill worker.

Well till the autumn of 1935, when he began to suffer from persistent tiredness and lassitude. During the last 5 to 6 weeks his condition became worse with striking rapidity, he lost his appetite completely and a little weight. He was up and about till eight days ago when he had to take to his bed. During the last two years periodic attacks of soreness of his tongue. He was admitted to the Med. Dept. A. of the Rikshospital 16—6—36.

*Present condition:* He is thin and his skin is pale-yellow. Sclerae jaundiced. Pulse 68, regular. Blood press. 120/60. Temp. 37.9. Pupils circular and equal, reacting to light and accommodation. Tongue smooth, moist, clean, pale. Cardiac dullness 4 c. to the left sternal margin. Apex beat fifth intercostal space, within the nipple line. Systolic murmur loudest over the apex. Urine: Schlesinger (1/10 dilut.) ++, conditions otherwise normal. Ewald test meal: (3/4 hr.) ac. 0/3, Congo ÷, McLean ÷. Wassermann ÷. Serum colour 14. S.R. 40 mm. after 1 hr. Hb. 19 % = 2.62 g. %. Hb. pr. erythr. 37 γγ. Erythrocytes 0.71 mill. Reticulocytes 21 ‰. Leucocytes 3000. Blood smear: myelocytes 3 %, young forms 2.5 %, band forms 3.5 %, polymorphs 55 %, monocytes 1 %, lymphocytes 35 %. Megalocytosis, mac-

Case 11. Cell count and Hmglob. from ear and sternal blood.

1936	time	Reticulocyt. per mille		Hmglob. pr erythrocyt. γγ		Hmglob.		Erythrocytes in millions		Nucleated blood cells		Erythroblasts in ear blood pr mm <sup>3</sup>
		ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	
16—6	12.—	—	—	34.5	—	17.—	—	0.68	—	—	—	—
	17.30	21	48	37.—	29.4	19.—	11.5	0.71	0.54	3000	5380	15.—
17—6	7.—	28	72	37.—	36.7	18.5	18.5	0.69	0.695	3000	43000	360.—
	17.—	62	84	35.5	22.2	18.—	4.5	0.70	0.28	4700	2660	—
18—6	7.—	75	116	33.9	19.4	18.5	4.0	0.77	0.29	5100	4800	470.—
	17.—	131	192	31.2	32.9	19.—	16.—	0.84	0.67	8900	39500	484.5
19—6	10.—	236	241	32.9	48.8	20.—	8.5	0.84	0.24	6500	3400	2269.5
	17.30	241	—	30.7	—	18.5	—	0.83	—	5900	—	1072.5
20—6	10.30	264	278	33.3	34.9	21.5	21.5	0.89	0.85	3700	30200	1111.5
	17.—	253	—	38.7	—	23.—	—	0.82	—	8800	—	171.—
21—6	18.—	155	—	38.4	—	26.—	—	0.935	—	4200	—	147.—
	18.—	110	—	37.4	—	24.5	—	0.94	—	4600	—	161.—
22—6		102	—	34.5	—	24.—	—	0.96	—	4100	—	82.—
23—6		56	—	41.1	44.1	27.5	10.—	1.005	0.336	4200	1120	21.—
26—6		23	—	36.—	—	29.—	—	1.19	—	5500	—	—
13—8		3	4	30.4	30.1	73.	68.—	3.32	3.12	7200	37100	—
7—9		1	1	26.9	24.—	88.—	54.—	4.51	3.10	5500	1900	—



rocytosis, poikilocytosis, Carbot's rings, anisocytosis, anisochromia, schizocytosis, microcytosis, polychromasia 4 ‰, erythroblasts 1/200 L. Marked over-segmentation of the nuclei of the neutrophil leucocytes.

*Sternal puncture:* megalomacroblastosis.

A radiological examination of the stomach and duodenum showed no other abnormality than slight gastritis.

*Treatment:* 16—6—36 20 cc. «Ei» «Nyco».

2—7—36 20 cc. «Ei» «Nyco».

13—7—36 40 cc. «Ei» «Nyco».

1—8—36 40 cc. Pernami «Nyco».

2—8—36 40 cc. Pernami «Nyco».

3—8—36 20 cc. Pernami «Nyco».

*Case 12. A. E. L. ♂ aged 59. Born 21—9—1875, Brandbu. (Ref. nr. 5249/36—37).*

*Occupation:* Ex-workman (Norsk Hydro).

Except for diarrhoea during the years 1905—1913, he was well until 1928 when he was said to have contracted influenza followed by renal disease with severe pain on micturition. Was in the Rjukan Hospital for seven months. Was then at work for a short time till, late in the autumn of 1929, he fainted suddenly while at work. He was admitted to the Rjukan Hospital where he remained for 4 to 5 months with the diagnosis of pernicious anaemia. He was put on a liver diet which he stuck to till Jan. 1935 when he ceased to eat liver. His tongue has often been sore, and he has experienced a burning sensation in his mouth and throat which have continued to be sore practically all the time since the autumn of 1929. Pricking and tingling in hands and feet since the summer of 1934.

On admission to the Med. Dept. A. of the Rikshospital 25—5—35, hb. 42 %, erythr. 2.4 mill. Serum colour 7. Under treatment with Pernami he made a good recovery, and after his discharge 27—7—35 he felt quite well for 3 to 4 months. Since then he has always felt listless, and his weakness during the last couple of months has been so marked that he has been obliged to lie down three or four times every day. He has eaten no liver, but has received some injections of Pernami. He was admitted on account of his growing lassitude to the Med. Dept. A. of the Rikshospital 28—12—1936.

*Present condition:* He is pale and drowsy, complaining of lassitude. Tongue smooth, atrophic, moist and clean. Pulse 60, regular. Temp. 37.6 Resp. 16, unembarrassed. Blood press. 100/60. Pupils circular, equal, reacting to light and accommodation. No demonstrable apex beat, no definite cardiac dulness, heart sounds weak. Spleen not palpable. Liver not enlarged, but with a suggestion of resistance just above the costal arch. Reflexes normal. Urine: Schlesinger (1/10 dilut.) +, in other respects no abnormality. Electrocardiogram normal. Wassermann ÷. Serum colour 7. S.R. (4—12) 35 mm. (During his previous stay in hospital, an Ewald test

meal showed Congo  $\div$ , McLean  $\div$ , ac. 0/3). Hb. 60.5 % = 8.349 g. %.  
 Hb. pr. erythr. 38.5  $\gamma\gamma$ . Erythr. 2.17 mill. Reticulocytes 5 %. Leucocytes  
 5800. Blood smear: bas. 0.5 %, eos. 2.5 %, myelocytes 0.5 %, band forms  
 3 %, polymorphs 47.5 %, monocytes 4 %, lymphocytes 42 %. Anisocytosis,  
 macro-megalocytosis, microcytosis, schizocytosis, poikilocytosis, poly-  
 chromasia, 1 per mille, orthochromia.

Case 12. Differential leucocyte count of ear blood.

1936—37		29—12	30—12	31—12	2—1	4—1	6—2
Praemyelocytes	per cent ....	—	—	—	0.5	1.—	—
Bas.	" " ....	0.5	0.5	0.5	—	—	0.5
Eos.	" " ....	2.5	1.5	3.—	2.5	2.5	—
Neutrophils	myelocytes	—	—	—	2.—	4.5	1.—
	young forms	0.5	—	1.—	3.5	2.5	4.—
	band forms	3.—	2.5	2.—	8.5	2.5	11.—
	polymorphs	47.5	51.5	42.5	47.—	40.5	50.—
Monocytes	" " ....	4.—	3.5	3.—	5.—	5.—	11.5
Lymphocytes	" " ....	42.—	40.5	48.—	31.—	41.5	22.—
Macrobl. » bas.	in 200 leu-	—	—	—	2	—	—
	eos. coeytes	—	1	1	1	1	—
Normobl. eos.	"	—	—	—	4	1	—

Case 12. Cell count and Hmglob. from ear and sternal blood.

1936— 1937	Reticulocytes per mille		Hmglob. per erythrocyte in $\gamma\gamma$		Hmglob.		Erythrocytes in millions		Nucleated blood cells		Erythro- blasts f- ear blood per mm.
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	
29—12	5	6	38.5	32.—	60.5	50.5	2.17	1.865	5800	21800	6.—
30—12	5	7	35.5	35.—	61.5	56.5	2.39	1.91	4500	20200	22.5
31—12	6	8	33.8	36.1	62.—	44.—	2.53	1.68	5200	57800	25.—
1—1	1										
2—1	44	72	33.4	35.4	61.5	51.—	2.54	1.99	8200	301600	257.—
3—1	67										
4—1	128	172	32.4	34.4	63.—	50.—	2.68	2.01	6800	65200	34.—
5—1	192										
6—1	121		32.6		61.—		2.58		7600		
7—1	103										
8—1	76	86	33.3	37.2	69.—	34.—	2.86	1.26	10200	17600	
11—1	16		34.2		68.—		2.74		8600		
13—1	8		35.2		62.—		2.43		9900		
5—2			32.8		95.—		4.00				

Case 12. Differential cell count of sternal blood.

1936—37		29—12	10—12	31—12	2—1	4—1	8—1
Sternal puncture		Asp. easy 0.8 cc. st. blood m.p. + + + f.p. + severe a. pain	Asp. easy 0.9 cc. st. blood m.p. + + + f.p. + severe a. pain	Asp. easy 0.5 cc. st. blood m.p. + + + + f.p. 0 severe asp. pain	Asp. under vac. [20 cc.] 0.5 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. under vac. [15 cc.] 0.35 cc. st. blood m.p. + + + + f.p. 0 severe asp. pain	Asp. easy 0.1 cc. st. blood m.p. + f.p. 0 moderate a. pain
Erythropoiesis	Erythroblasts per cent	26.8	22.25	43.—	54.—	37.—	21.—
	Leuco- blasts	73.2	77.75	57.—	46.—	63.—	79.—
	Promegalomacroblasts	3.5	10.—	0.50	0.3	1.—	—
	Megalobl. bas.	12.—	9.—	0.50	1.—	—	—
	eos.	—	—	—	—	1.—	—
	Macrobl. bas.	51.5	57.—	88.50	14.3	21.—	32.—
	eos.	23.—	13.—	1.—	—	5.5	12.—
	Normobl. bas.	—	—	2.—	80.—	40.5	8.—
	eos.	—	—	1.50	3.7	25.5	28.—
	Erythrobl. bas.	2.5	2.—	1.—	—	1.5	8.—
Leucopoiesis	eos.	7.5	9.—	1.50	—	2.—	12.—
	Erythrobl. bas.	—	—	0.50	0.7	1.—	—
	division forms eos.	—	—	—	—	—	—
	Megacaryocytes	0.50	—	—	—	—	—
	Mast cells immature	0.50	—	0.75	0.25	0.50	1.—
	mature	—	—	—	—	—	—
	Eos. myelocytes	0.50	0.75	1.—	1.25	1.—	2.—
	leucocytes band forms	1.—	0.50	0.25	2.50	0.25	1.—
	polymorphs	0.50	1.—	1.—	0.25	0.75	2.—
	Myeloblasts	4.25	1.50	4.25	1.75	1.75	1.—
	Praemyelocytes	4.25	4.—	4.50	2.50	5.25	1.—
	myelocytes	16.75	15.—	22.75	26.75	16.75	15.—
	Neutro- young forms	22.50	16.—	27.25	24.75	29.—	38.—
	philes band forms	9.—	5.25	7.—	7.50	15.25	12.—
	polymorphs	19.75	25.50	5.50	1.25	15.50	18.—
	Mono- blasts	—	—	—	—	—	—
	cytes	2.25	1.25	1.75	1.—	1.75	1.—
	Lymphocytes	6.—	17.50	1.—	0.75	2.75	2.—
	Plasma & Türk cells	0.25	—	—	0.25	0.50	—
	Reticulo endothel. cells	1.25	0.25	0.25	—	—	—
	Smear cells	10.50	11.50	22.75	29.25	9.—	6.—
	Unclassified cells	0.25	—	—	—	—	—



*Sternal puncture:* megalo-macro-blastosis. A radiological examination of the stomach and duodenum showed normal conditions.

*Treatment:* 29—12—36 4 cc. Mrk. (x)<sup>E</sup> = 10 mm. dried substance  
 12—1—37 20 cc. Mrk. E<sup>b</sup> Ph. = 1000 g. liver.  
 28—1—37 10 cc. Mrk. E<sup>b</sup> Ph. = 500 g. liver.

*Case 13. T. E. ♂ aged 25. (Ref. nr. 6594/35—36).*

*Occupation:* Agent.

Healthy till October 1929 when, 19 years old, he began gradually to feel tired, relaxed and short of breath, losing his appetite. He turned a pale yellow, and a little later his tongue felt sore, and he vomited his food whatever its nature. Admitted to the Bårum Hospital, he was given liver treatment. In December, 1931, he was admitted again to the Bårum Hospital for the same symptoms, and now he was almost comatose. Hb. 20 %, erythr. 0.565 mill., leucocytes 1200. Serum clour 34. Blood transfusion and liver treatment, the patient being discharged perfectly well. About Christmas 1935 he began to grow steadily worse, lassitude and breathlessness on exertion increasing. During the last 4 or 5 days diarrhoea and increasing pallor. Admitted to Med. Dept. A. of the Rikshospital 13—2—1936.

Case 13. Differential leucocyte count of ear blood.

1936		13—2	17—2	18—2	19—2	20—2	21—2	22—2	4—3
Bas.	per cent	—	—	—	—	—	0.5	—	—
Eos.	» »	1	2	4	3.—	3.5	2.5	0.5	2.5
Neutrophils	myelocytes	—	11	8	7.—	11.0	6.5	2.5	—
	young forms	1	—	9	8.—	13.5	4.0	2.0	1.0
	band forms	1	8	6	12.—	14.0	3.5	3.5	3.0
	polymorphs	13	30	31	42.—	28.0	35.5	44.0	55.0
Monocytes	» »	3	4	3	0.5	0.5	3.0	2.5	1.0
Lymphocytes	» »	81	45	39	27.5	29.5	44.0	44.5	37.5
Plasma-Türk	» »	—	—	—	—	—	0.5	0.5	—
Megalobl. bas.	in 200 leucocytes	—	—	1	—	5	—	—	—
		—	—	—	1	1	—	—	—
Normobl. bas.	»	—	—	—	10	21	2	1	—
		—	—	—	2	58	26	11	—
Macrobl. bas.	»	—	3	1	5	8	1	1	—
		—	—	1	—	9	—	—	—
Erythrobl. bas.	»	—	1	—	1	—	—	—	—
Erythrobl. Division forms	bas. »	—	—	—	—	1	—	—	—

Case 13. Cell count and Hmglb. from ear and sternal blood.

1936	Reticulocytes per mille		Hmgb. pr. erythrocyt. in %		Hmglb.		Erythrocytes in millions		Nucleated blood cells		Erythro- blasts in ear blood pr. mm <sup>3</sup>
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	
8-2	1		26.8		52.5		2.705				
10-2	2		25.4		43.5		2.36				
13-2	3		26.2		40.5		2.13		2800		
14-2	5		27.6		40.—		2.00		4000		
15-2	5		27.7		40.—		1.99				
16-2	6						1.86				
17-2	4	18	28.1	24.7	41.5	23.5	2.01	1.31	4200	123700	168.—
18-2	4	23	30.—	31.9	41.5	37.5	1.91	1.62	4300	216000	129.—
19-2	1	21	30.5	30.6	41.5	37.5	1.88	1.69	7100	82060	674.5
20-2	68	90	30.3	27.0	40.—	25.—	1.82	1.28	5800	6180	2978.—
21-2	140	376	28.6	29.1	42.5	40.50	2.03	1.92	6600	224000	957.—
22-2	135	332		36.1	47.—	42.—		1.92	5200	120500	338.—
24-2	81		33.5		53.—		2.15		8200		
26-2	32		33.2		53.—		2.20		6300		
29-2	5		34.2		55.—		2.22		8300		
2-3	8	14	31.9	36.8	52.—	44.5	2.25	1.67	5900	45200	
4-3	14	14	32.1	36.9	47.—	45.—	2.02	1.68	5300	117200	
6-3	146		32.1		56.—		2.41		5000		
7-3	219										
8-3	258										
9-3	111		30.1		60.—		2.75		4700		
22-3	2		28.8		70.—		3.86		4900		
30-3	20		26.—		83.—		4.42		7900		

*Present condition:* He is pale and thin, mucous membranes pale, slight jaundice of the skin. Temp. 38. Tongue moist, clean, smooth. Left eye blind after an injury when a boy. The pupil of the right eye small and with a somewhat angular and irregular outline. The iris slightly blurred, opacities in the cornea. His pupils react well to light, less well to accommodation. Before they were drawn, his teeth were said to have been "queers" — probably Hutchinson's teeth. Liver not palpable. Blood press. 120/60. Parenchymatous keratitis diagnosed at the Eye Dept. Reflexes normal. No anaesthesias or paraesthesias. Ewald test meal: ac. 0/5, Congo ÷, McLean ÷. Urine: Schlesinger (1/10 dilut.) ++, Wassermann ÷. Serum colour 8. S.R. after 1 hr. 14 min. Reticulocytes 3‰. Hb. 40.5 % = 5.59 g. %. Hb. pr. erythr. 26.2%. Erythr. 2.13 mill. Leucocytes 2800. Blood smear: eos. 1 %, metamyelocytes 1 %, band forms 1 %, polymorphs 13 %, lymphocytes 81 %, monocytes 3 %. Marked anisocytosis, microcytosis, schizocytosis, poikilocytosis megalocytosis, macrocytosis and polychroma-

Case 13. Differential cell count of sternal blood.

1936			17—8	18—2	19—2	20—2	21—2	22—2	2—3	4—3
Erythropoiesis	Erythroblasts	per cent	34.6	33.—	49.2	13.—	47.2	25.4	29.2	63.8
	Leuco- blasts	»								
	cytes	»	65.4	67.—	50.8	87.—	52.8	74.6	70.8	36.2
	Promegalomacroblasts	»	31.7	31.6	1.—	—	0.4	0.8	3.4	—
	Megalobl. bas.	»	9.2	11.4	1.60	—	3.4	—	45.5	5.8
	cos.	»	—	1.8	1.7	7.7	2.1	0.8	12.4	2.4
	Macrobl. bas.	»	13.3	25.2	41.9	11.6	29.5	14.9	—	11.8
	cos.	»	—	—	—	—	—	1.—	—	—
	Normobl. bas.	»	—	3.0	11.7	30.8	20.2	14.9	1.3	65.8
	cos.	»	—	0.6	—	19.3	26.—	20.4	1.3	7.1
	Erythrobl. bas.	»	44.6	14.4	40.5	—	5.6	13.4	19.2	0.7
	cos.	»	—	6.—	—	30.6	12.—	30.9	15.8	1.9
	Erythrobl. bas.	»	1.2	6.—	1.6	—	0.8	3.9	1.3	3.8
	division forms Oos.	»	—	—	—	—	—	—	—	0.7
Leucopoiesis	Megacaryocytes	»	—	—	—	—	—	—	—	0.3
	Mast cells immature	»	—	—	0.50	—	0.25	0.50	0.75	0.7
	mature	»	—	—	0.50	—	—	—	—	—
	Eos. myelocytes	»	3.5	1.50	2.50	—	4.25	1.25	1.—	4.3
	leucocytes band forms	»	1.75	1.50	1.00	0.5	2.50	2.50	4.75	4.—
	polymorphs	»	1.50	01.50	1.25	2.5	1.25	2.25	2.50	1.3
	Myeloblasts	»	0.25	0.50	0.75	—	0.75	0.50	1.—	2.3
	Praemyelocytes	»	3.—	3.75	5.25	7.—	3.—	3.75	5.75	3.7
	Neutro- myelocytes	»	19.—	20.25	16.75	5.—	10.—	4.75	8.50	18.—
	philes young forms	»	32.50	29.—	32.25	6.—	19.25	17.25	13.—	19.3
	band forms	»	25.—	28.50	29.—	18.—	42.—	41.—	30.—	31.—
	polymorphs	»	6.5	4.50	8.75	31.—	9.75	20.—	23.75	12.—
	Mono- blasts	»	—	—	—	2.5	0.50	—	1.25	0.3
	cytes	»	—	—	—	—	—	—	—	—
	Lymphocytes	»	1.—	1.50	1.—	27.5	1.—	4.25	5.75	1.7
	Plasma & Türk cells	»	0.25	0.25	—	—	—	0.50	0.25	0.7
	Reticulo endothelial cells	»	4.5	6.75	0.5	2.—	5.50	0.75	1.25	0.3
	Smear cells	»	—	—	—	—	—	—	—	—
	Leucocytes divisions forms	»	1.25	0.50	—	—	—	0.75	—	—

sia. Basophil punctuation. No over-segmentation of the nuclei of the neutrophil cells.

*Sternal puncture:* megalomacro-blastosis.

*Treatment:* 17—2—36 2.5 cc. B-bu-perf. Bu = phase = 300 g. liver.

2—3—36 6 cc. B-bu-perf. Bu = phase = 750 g. liver.

24—3—36 3 cc. Merk. E.

Diabetes mellitus was diagnosed in 1941.

Case 14. Differential leucocyte count of ear blood.

1936	28-4	29-4	30-4	1-5	2-5	4-5	5-5	6-5	7-5	8-5	9-5	11-5	12-5	15-5
Præmyelocytes per cent	0.5	0.5	0.5	--	--	--	--	--	--	--	--	--	--	--
Bas.	2.0	0.5	0.5	--	0.5	1.0	--	--	--	1.5	--	--	0.5	0.5
Eos.	0.5	--	--	--	0.5	0.5	0.5	--	0.5	0.5	--	0.5	1.0	0.5
Neutrophils { myelocytes young forms band forms polymorphs	1.0	1.5	1.5	1.5	--	0.5	--	--	--	--	0.5	--	--	--
	1.0	2.5	0.5	3.0	0.5	0.5	--	--	0.5	0.5	0.5	0.5	0.5	0.5
	5.5	5.0	4.0	4.0	4.5	4.0	4.5	4.5	2.0	6.0	3.5	4.5	4.0	3.5
	44.0	41.5	46.0	43.0	37.0	47.0	55.0	55.0	64.0	46.0	41.0	51.5	46.5	56.0
Monocytes	--	1.0	1.0	2.0	2.0	--	1.0	2.5	0.5	1.5	0.5	0.5	0.5	2.0
Lymphocytes	46.0	47.5	46.0	46.5	55.0	46.5	39.0	37.0	32.5	44.0	53.0	42.0	47.0	37.0
Megalobl. bas. in 200	1	1	--	1	1	--	--	--	--	--	--	--	--	--
cos. leucocyt.	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Normobl. cos.	--	--	6	5	--	1	--	--	--	--	--	--	--	--
Macrobl. bas.	2	4	2	1	--	--	2	1	--	--	--	--	--	--
cos.	1	--	1	1	--	1	1	--	1	--	--	--	--	--
Erythrobl. bas.	--	--	1	--	--	--	--	--	--	--	--	--	--	1
cos.	1	--	1	2	--	--	--	--	--	1	--	1	--	1

Case 14. I. J. ♀ aged 56. (Ref. nr. 8775/35—36).

At the age of 30, severe catarrh of the intestines, confined to bed for three months. After this illness her abdomen never functioned as before, and she was always liable to suffer from diarrhoea. At Christmas 1935 increasing lassitude, tiredness, disinclination for work. Breathless on walking up hill or up stairs. Subject to a sense of pressure in her head, headache, giddiness, tinnitus. Became gradually more pale, and turned so yellow that her neighbours thought she had jaundice. Declining appetite. Paraesthesias of hands and feet from time to time. Early in 1936 soreness of her tongue for 8 days. Consulted a doctor early in March, 1936 for influenza which lasted 3 weeks. Was thereupon drafted through the Medical Polyclinic of the Rikshospital to its Med. Dept. A. 24—4—1936. Appetite poor latterly, lost 11 kg. in the course of 3 years.

*Present condition:* Skin pale yellow, general state of nutrition good, no complaints. Pulse 98, regular, rather weak. Resp. 24, unembarrassed. Temp. 38.5. Tongue pale, moist, slightly coated, somewhat enlarged papillae on its central part, the rest of it being smooth. Blood press. 120/65. Sclerae subicteric, no enlargement of the glands. Liver palpable 3 finger-breadths below the right costal arch. Chest findings otherwise normal apart from a blowing systolic murmur over the whole of the heart. Ewald test

Case 14. Cell count and hmglob. from ear and sternal blood.

1936	Reticulocytes pr. mille		Hmglob. pr. erythrocyt. in %		Hmglob.		Erythrocytes in millions		Nucleated blood cells		Erythro- blasts in ear blood pr. mm <sup>3</sup>
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	
27—4	9	17	40.6	36.7	35.—	33.5	1.19	1.26	2200	74800	—
28—4	12	18	38.6	43.7	35.5	34.5	1.27	1.09	3600	99900	90.
29—4	16	32	37.6	41.5	36.—	34.0	1.32	1.13	4100	49900	103.—
30—4	24	37	42.—	37.7	35.—	30.—	1.15	1.10	4400	156000	242.—
1—5	46	80	39.3	46.1	37.5	38.5	1.31	1.13	4500	80300	225.—
2—5	161	217	40.—	40.—	40.—	38.—	1.39	1.31	4900	12750	24.—
4—5	168	—	42.4	—	43.—	—	1.40	—	3300	—	33.—
5—5	72	80	42.5	40.—	49.—	44.—	1.59	1.52	5800	40600	87.—
6—5	62	—	41.3	—	44.—	—	1.47	—	4500	—	22.—
8—5	24	15	43.6	37.—	45.5	46.—	1.44	1.35	3100	9500	15.—
9—5	26	—	41.7	—	42.—	—	1.39	—	3000	—	0.—
11—5	30	29	41.5	44.1	44.5	46.—	1.48	1.42	3200	58500	16.—
12—5	34	—	39.5	—	45.—	—	1.57	—	—	—	0.—
14—5	12	—	42.2	—	44.—	—	1.44	—	4200	—	—
15—5	15	12	39.7	36.3	45.5	31.—	1.58	1.18	2800	11700	23.—
19—5	13	—	38.9	—	42.—	—	1.49	—	4200	—	—
16—6	3	—	34.8	—	73.—	—	2.89	—	6600	—	—

Case 14. Differential cell count of sternal blood

1936		27—6	28—4	29—4	30—4	1—5	2—5	5—5	8—5	11—5	15—5
Sternal puncture		Asp. easy 0.9 cc. st. blood m.p. ++ f.p. 0	Asp. easy 0.75 cc. st. blood m.p. ++ f.p. 0	Asp. easy 1.1 cc. st. blood m.p. ++ f.p. 0	Asp. easy 0.6 cc. st. blood m.p. ++ f.p. 0	Asp. easy 0.6 cc. st. blood m.p. + f.p. 0	Asp. und. vac. [20 cc.] 0.2 cc. st. blood m.p. (+) f.p. 0	Asp. easy 0.5 cc. st. blood m.p. + f.p. 0	Asp. under vac. 0.4 cc. st. blood m.p. 0 f.p. +	Asp. easy 0.55 cc. st. blood m.p. + f.p. 0	Asp. under vac. 1 cc. st. blood m.p. 0 f.p. 0
Erythroblasts	per cent	38. —	59.4	55.3	66.4	62.8	35. —	37.2	17. —	42. —	19.6
Leuco blasts	"	62. —	40.6	44.7	33.6	37.2	65. —	62.8	83. —	58. —	83.4
cytes	"	—	1. —	—	3. —	—	—	—	—	—	—
Promegalomacroblasts	"	—	—	—	1.2	—	1.1	4.1	4.7	5.2	4. —
Megalobl.	bas.	10. —	2. —	0.3	—	—	8.6	40.4	2.3	1. —	—
Macrobl.	cos.	33.7	39.2	62.5	49.9	10.8	—	—	16.6	37.6	22. —
bas.	"	6.8	—	3.9	2.4	0.6	9.2	1.1	1.2	4.8	3. —
cos.	"	—	—	3.6	12.1	59. —	6.3	21.6	15.4	13.3	23. —
bas.	"	—	0.7	2.4	8.2	24. —	—	11.3	—	5.2	27. —
cos.	"	22.6	38.6	14.1	6.6	—	11.1	10.8	14.4	18.4	9. —
Erythrobl.	bas.	24.2	17.5	10.5	12.1	4.7	33.7	8.6	15.4	11.9	10. —
Erythrobl.	cos.	1.1	1. —	2.4	1.8	0.6	—	2.1	—	2.4	2. —
division forms	"	—	—	—	2.7	0.3	—	—	—	0.5	—
Megacaryocytes	"	—	—	—	0.25	0.50	—	0.25	—	—	—
Mast cells	immature	0.25	0.50	—	0.25	0.25	—	—	—	—	—
mature	"	—	—	—	—	—	—	—	—	—	—
Eos- myelocytes	"	0.75	1.25	1.25	3.50	0.75	0.75	0.25	0.25	0.50	0.50
leuco- band forms	"	0.25	1.25	0.25	1. —	0.75	1.50	1. —	1. —	2. —	0.25
cytes polymorphs	"	0.75	0.50	—	0.25	0.50	1.75	0.50	0.75	0.50	0.50
Myeloblasts	"	1.25	0.50	0.25	1. —	2.25	0.50	1. —	0.75	1.50	1. —
Praemyelocytes	"	2.25	3.25	3. —	5.75	4. —	1.75	1.50	1. —	3.50	1.25
Neu- myelocytes	"	18.75	19. —	14.25	14.50	17.50	1.50	11.50	5. —	10. —	6.75
tro- young forms	"	20. —	29.50	12.75	27.50	21.75	14.50	22.75	5.75	16.75	14.50
philes band forms	"	21. —	18.75	17.75	13.25	14.25	11.75	16.25	5.75	17. —	10.75
polymorphs	"	9.75	9.25	18.50	12.50	18.25	26. —	23.50	44.25	18.75	26. —
Mono- blasts	"	—	—	—	—	—	—	—	—	—	—
cytes	"	0.50	0.75	0.75	0.25	0.75	0.25	1.75	0.25	1. —	1.25
Lymphocytes	"	14.75	6. —	13.25	1.75	6.25	28.75	10.75	22.50	16. —	30. —
Plasma & Türk cells	"	—	0.25	—	—	—	0.50	—	—	—	—
Reticulo endothel. cells	"	0.25	0.75	0.25	0.50	0.50	—	—	0.25	0.75	—
Smear cells	"	—	8.50	17.75	17.75	11.75	7.50	9. —	12.50	11.75	7.25

meal: ac. 0/10. Wassermann  $\div$ . Urine: Schlesinger  $\div$ , otherwise normal. Serum colour 9. Hb. 35 % = 4.83 g. %. Hb. pr. erythr. 40.6  $\gamma\gamma$ . Erythr. 1.19 mill. Leucocytes 2200. Reticulocytes 9  $\frac{1}{100}$ . Blood smear: bas. 0.5 %, eos. 2 %, myelocytes 1 %, metamyelocytes 1 %, band forms 5.5 %, polymorphs 44 %, lymphocytes 46 %, Polychromasia, basophil punctuation, anisocytosis, orthochromia, macrocytosis, megalocytosis, schizocytosis, microcytosis, erythroblasts 5/200 L.

*Sternal puncture:* megalomacroblastosis.

*Treatment:* 27—4—36 4 cc. BBaBF. u.s. E = 200 g. liver.

2—6 & 3—6 altogether 40 cc. Pernami «Nycos».

11—5 4 cc. BBaBF. u. s.-Bentz — ads. = 200 g. liver.

*Case 15. J. A. J. ♂ aged 52. Born in V. Moland. (Ref. nr. 9176/35—36.)*

*Occupation:* Steward.

Suffered from rheumatic fever in 1905 and 1927. Appendicectomy in 1925, gonorrhoea in 1916. Stricture of the urethra in 1934. A fortnight ago much blood in the urine one morning. From time to time slight soreness of the tip of the tongue, and during the last 3 weeks slight paraesthesias of his fingers. His complexion has become pale brown, and his conjunctivae have become rather pale. Admitted to the Med. Dept. A. 7—5—36.

*Present condition:* He is pale. Puls 80, regular. Blood press. 130/80. Temp. 37.3. Tongue moist, clean, and smooth, particularly over its borders. There are a few slightly swollen and injected papillae on the tip of his tongue. Resp. 16, unembarrassed. Findings of physical examination normal. Urine: acid, cloudy, sp. gr. 1015, alb.  $\div$  no pus, guaiac  $\div$ , Bene-

Case 15. Differential leukocyte count of ear blood.

1936				12—5	13—5	19—5	20—5	22—5	23—5
Bas.	per cent	....		0.5	0.7	—	—	0.5	—
Eos.	»	»	....	3.5	4.3	4.0	4.0	3.5	4.0
Neutrophils	young forms	»	....	0.5	—	—	—	—	—
	band forms	»	....	2.0	3.0	6.5	4.0	5.5	6.0
	polymorphs	»	....	26.0	41.7	48.5	41.5	42.0	61.7
Monocytes	»	»	....	4.5	2.3	4.0	3.0	4.5	5.3
Lymphocytes	»	»	....	63.0	48.0	37.0	47.5	44.0	33.0
Macrobl.	bas. in 200 leucocytes			—	—	1	—	—	—
Erythrobl.	eos.	»		—	—	1	—	—	—
	bas.	»		—	—	—	—	1	—

Case 15. Cell count and hmglob. from ear and sternal blood.

1936	Reticulocytes per mille		Hmgb. pr. erythroc. in $\gamma\gamma$		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
13—5	7	8	46.4	45.2	80.—	62.5	2.38	1.91	4100	44900
18—5	3									
19—5	7	12	43.3	34.8	82.5	63.—	2.63	2.50	4300	49300
20—5	2	3	45.2	40.7	78.—	70.5	2.38	2.39	4600	82100
21—5	7		29.4		72.—		2.52			
22—5	10	17	41.3	40.8	77.—	71.5	2.57	2.42	5100	70000
23—5	28	36	38.2	50.7	75.5	72.—	2.72	1.96	5700	46600
24—5	24									
25—5	37									
26—5	44									
27—5	18									
28—5	11		39.2		78.—		2.74		7600	
15—7			29.—		90.—		4.28		7100	

dict ÷, numerous leucocytes and erythrocytes and b. coli. The urine yields b. coli on culture. No tubercle bacilli in a 24-hr. sample of urine, nor on culture of catheter urine. Ewald test meal: (after 3/4 hr.) ac. 0/4, Congo ÷ McLean ÷. Wassermann ÷. Gon. comp. fix. ÷. Serum colour 4. Hb. 80 % = 11.04 g. %. Hb. pr. erythr. 46.4  $\gamma\gamma$ . Erythrocytes 2.38 mill. Reticulocytes 7‰. Leucocytes 4100. Blood smear: eos. 3.5 %, bas. 0.5 %, metamyelocytes 0.5 %, band forms 2 %, polymorphs 26 %, monocytes 4.5 %, lymphocytes 63 %. Anisocytosis, macrocytosis (probably also megacytosis) faint anisochromia, schizocytosis and microcytosis. No polychromasia nor poikilocytosis. Faint signs of oversegmentation of the nuclei of the neutrophil cells.

*Sternal puncture:* megalomacroblastosis.

Between 12—6—36 and about 5—7—36 the patient suffered from acute rheumatism during which he seemed to be less amenable to liver treatment. A radiological examination after intravenous pyelography showed nothing amiss apart from prostatic calculi. A cystoscopic examination showed no ulcers, tubercles or concretions.

*Treatment:* 18—5—36 10 cc. Mrk. Bentz. filtr. = 500 g. liver.

2 & 3—6—36 a total of 40 cc. Pernami.

26/30—6—36 a total of 100 cc. Pernami.



Case 15. Differential cell count of sternal blood.

1936		13—5	19—5	20—5	22—5	23—5
Sternal puncture		Asp. easy 0.4 cc. st. blood m.p. + + f.p. 0	Asp. easy 0.35 cc. st. blood m.p. + f.p. 0	Asp. easy 0.35 cc. st. blood m.p. + + f.p. +	Asp. easy 0.5 cc. st. blood m.p. + + f.p. +	Asp. easy 0.45 cc. st. blood m.p. + f.p. +
Erythropoiesis	Erythroblasts per cent	24.—	18.8	33.6	41.—	51.7
	Leuco- blasts »	76.—	71.2	66.4	59.—	48.3
	cytes »	—	—	—	—	—
	Promegalomacroblasts »	—	—	—	—	—
	Megalobl. bas. »	2.6	4.3	0.6	—	—
	eos. »	—	—	0.6	1.—	—
	Macrobl. bas. »	13.5	35.—	19.—	12.2	7.—
	eos. »	1.7	8.5	1.2	11.7	4.—
	Normobl. bas. »	26.2	10.6	58.8	22.—	7.—
	eos. »	17.8	9.6	9.6	43.8	78.5
	Erythrobl. bas. »	27.9	6.4	1.8	1.—	1.—
	eos. »	9.4	22.3	5.4	6.3	2.—
	Erythrobl. bas. »	0.9	3.2	3.—	1.—	—
	division forms eos. »	—	—	—	1.—	0.5
Leucopoiesis	Megacaryocytes »	—	—	—	—	—
	Mast cells immature »	0.50	0.50	0.25	0.25	—
	mature »	—	0.25	—	—	—
	Eos. myelocytes »	3.25	1.50	3.25	2.75	1.7
	leucocytes band forms »	2.—	2.—	3.75	1.50	1.80
	polymorphs »	1.25	2.75	2.25	1.75	1.3
	Myeloblasts »	2.75	1.—	1.25	2.25	0.3
	Praemyelocytes »	2.75	2.25	4.25	3.25	1.7
	myelocytes »	17.75	7.25	18.75	14.75	25.7
	Neutro- young forms »	23.75	16.25	23.25	25.50	22.—
	philes band forms »	17.25	23.75	24.75	12.50	18.7
	polymorphs »	14.25	15.25	10.50	10.—	13.7
	Mono- blasts »	} 0.50	2.—	0.25	1.—	—
	cytes »		—	—	—	—
	Lymphocytes »	8.75	13.50	3.75	7.75	6.—
	Plasma & Türk cells »	0.75	1.—	1.06	—	1.—
	Reticulo endothelial cells »	—	—	—	—	0.3
	Smear cells »	4.50	9.50	2.75	16.75	—

Case 16. J. S. ♀ aged 48. Born 6—11—1888, Svindal (Ref. nr. 7038/36—37).

Occupation: Wife of painter.

Since the age of 7 or 8 she has suffered from dyspepsia which her doctors regarded as nervous. During the last 10 years, since 1927, she has suffered from breathlessness. Fully a year ago she began to experience after every meal a stinging, burning pain in the epigastrium about  $\frac{1}{2}$  hr. after food. Eating eased this pain. In March 1935, a doctor discovered anacidity, and in July 1936, pernicious anacmia was diagnosed. (Haemoglobin 45 % July 20.). After eating liver for 4 to 5 weeks, she could stand it no longer. During the past two years she has suffered from growing lassitude, but she stuck to her work till admitted to hospital. A couple of years ago her tongue and roof of her palate were so sore for a couple of weeks that she experienced difficulty in eating. A year ago the base of her tongue was sore for some days. She was liable to diarrhoea after fat food, and she lost much weight during the last two years. An examination 17—2—1937 at the Bethanien medical laboratory showed haemoglobin 54 %, 2.26 million erythrocytes, and 5100 leucocytes. She was accordingly admitted 24—2—1937 to the Med. Dept. A. of the Rikshospital.

*Present condition:* She is pale and her mucous membranes are pale. State of general nutrition moderate. Temp. 38.2. Tongue moist, clean, smooth. Resp. unembarrassed. Pulse 84, regular. Blood press. 115/60. Liver and spleen not palpable. Reflexes normal. No oedema. Electrocardiogram normal. Ewald test meal ( $\frac{3}{4}$  hr.) Congo  $\div$ , McLean  $\div$ , ac. 0/3. Urine: Schlesinger (1/10 dilut.) ++. Wassermann  $\div$ . Serum colour 6. S.R. (after 1 hr.) 20 mm. Retics. 43 %<sub>00</sub>. Hb. 70 % = 9.66 g. %. Hb. pr. erythr. 43.9 %<sub>00</sub>. Erythr. 2.20 mill. Leucocytes 6200. Blood smear: eos. 4.5 %, band forms 2 %, metamyelocytes 0.5 %, polymorphs, 46.5 %, monocytes 3.5 %, lymphocytes 43 %. Anisocytosis, macro-megalocytosis, microcytosis, schizocytosis, orthochromia, polychromasia < 1 %<sub>00</sub>, over-segmentation of the nuclei of the neutrophil cells. Nothing abnormal found on a radiological examination of stomach and bulbus duod.

*Treatment:* 1—3—1937 2 cc. Mrk. (X)<sup>E</sup> = I ing.t.s.

13—3—1937 20 cc. Pernami

18—3—1937 20 cc. Pernami

23—3—1937 20 cc. Pernami

28—3—1937 20 cc. Pernami

2—4—1937 20 cc. Pernami

6—4—1937 20 cc. Pernami

Case 16. Differential leucocyte count of ear blood.

1937				25—2	1—3	3—3	5—3	10—3
Bas.	per cent	.....		—	—	—	—	0.5
Eos.	»	»	.....	2.0	4.5	2.0	1.5	2.0
Neutrophils	{ myelocytes	»	.....	—	—	—	0.5	—
	{ young forms	»	.....	—	0.5	—	0.5	—
	{ band forms	»	.....	3.5	2.0	4.0	2.5	2.0
	{ polymorphs	»	.....	65.5	46.5	51.5	57.0	60.5
Monocytes	»	»	.....	3.0	3.5	1.0	2.0	1.5
Lymphocytes	»	»	.....	26.0	43.0	41.5	36.0	33.5
Macrobl. bas. in 200 leucocytes				—	—	—	—	—
eos.				—	—	—	1	—

Case 16. Cell count and Hmglb. from ear and sternal blood.

1937	Reticulocytes per mille		Hmgb. pr. erythrocyt. in %		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
25—2	43		43.9		70		2.20		6200	
27—2	35									
28—2	36									
1—3	29	38	42.9	42.9	64	60	2.00	1.93	4000	66000
2—3	26									
3—3	28	34	42.7	43.6	68	72	2.20	2.28	4800	132000
4—3	53									
5—3	42	88	44.4	31.1	72	67	2.24	1.81	4300	140000
6—3	95									
7—3	82									
8—3	90	91	43.5	46.—	70	69	2.22	2.07	5500	66700
9—3	99									
12—3	44		44.5		68		2.11		4000	
17—3	274		43.5		70		2.22		4800	
20—5	168		38.8		72		2.56		3900	
24—3	50		35.8		84		3.27		7800	
3—4	34		30.8		85		3.81			

Case 16. Differential cell count of sternal blood.

1937				1—3	3—3	5—3	8—3
Sternal puncture				Asp. easy 0.6 cc. st. blood m.p. + + f.p. 0 severe a. pain	Asp. easy 0.2 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. easy 0.7 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. easy c. 8 cc. st. blood m.p. + + + f.p. 0 severe a. pain
Erythropoiesis	Erythroblasts		per cent	22.9	33.3	31.3	20.—
	Leuco-	blasts	»				
		cytes	»	77.1	66.7	68.7	80.—
	Promegalomacrobasts		»	—	—	3.—	3.—
	Megalobl.	bas.	»	5.—	—	2.—	2.—
		eos.	»	—	—	—	—
	Macrobl.	bas.	»	62.—	52.5	35.—	63.—
		eos.	»	4.—	5.—	7.—	13.—
	Normobl.	bas.	»	10.—	19.—	8.—	5.—
		eos.	»	7.—	17.—	38.—	3.—
	Erythrobl.	bas.	»	2.—	1.5	1.—	3.—
		eos.	»	5.—	2.5	2.—	8.—
	Erythrobl.	bas.	»	5.—	2.5	4.—	—
	division forms	eos.	»	—	—	—	—
Leucopoiesis	Megacaryocytes		»	+	+	+	—
	Mast cells	immature	»	0.25	0.25	0.50	0.25
		mature	»	—	—	—	—
	Eos.	myelocytes	»	1.25	0.75	1.50	2.25
	leucocytes	band forms	»	1.—	1.75	1.50	3.—
		polymorphs	»	1.—	0.75	1.25	2.—
	Myeloblasts		»	2.75	2.25	1.75	2.—
	Praemyelocytes		»	4.—	3.75	2.75	3.75
	Neutro-	myelocytes	»	15.25	11.50	20.25	15.25
	philes	young forms	»	22.50	22.75	27.50	24.25
		band forms	»	14.—	16.75	11.75	12.50
		polymorphs	»	15.—	13.50	9.50	14.25
	Mono-	blasts	»				
		cytes	»	1.25	1.—	1.—	0.75
	Lymphocytes		»	13.25	15.50	10.75	12.50
	Plasma & Türk cells		»	—	0.75	0.25	—
	Reticulo endothelial cells		»	0.25	—	0.25	—
	Smear cells		»	8.25	8.75	9.50	7.50

*Case 17. S. T. ♂ aged 72. Born in Sweden. (Ref. nr. 3124/36—37)*

*Occupation: Ex-mason.*

Well till June 1933 when he began to suffer from thirst, polyuria, dyspepsia and nausea. He lost 5—6 kg. during the summer, sought medical aid, and was admitted in July 1933 to the Med. Dept. A. of the Rikshospital for diabetes mellitus. Incipient cataract was diagnosed at the Eye Dept. He was discharged on a dietary of 2500 calories without insulin. In April 1935 he began again to be troubled by lassitude, anorexia, headache, dimness of vision, flickering eyesight, thirst and polyuria. On re-admission to the Med. Dept. A. 6—5—35, his tongue was moist, clean, and very red. Ewald test meal (3/4 hr.) ac. 0/8, Congo ÷ McLean ÷. Wassermann ÷, blood press. 150/80, Hb. 83 %, erythr. 4.27 mill. Leucocytes 6000. Discharged on a mixed diabetes dietary with 4 + 7 units insulin. From July 1936 he began to suffer from lassitude, oedema of the feet up to the knees, breathlessness worst on walking up hill. In the middle of August, attacks of vomiting unrelated to meals. In September, increase of the oedema, lassitude and breathlessness. A doctor found he was suffering from dropsy and lack of blood, prescribing rest in bed and iron. The oedema passed off, but the lassitude and dyspnoea increased. On Oct. 19, suffocating pain in the epigastrium changing to colic lasting altogether 3 hours. Since his previous stay in hospital has lost 7 kg. Has not suffered from soreness of his tongue, but during the last few months has been troubled by some pricking in the fingers. Constipated. Re-admitted to the Med. Dept. A. 15—10—36.

*Present condition:* He gives an anaemic impression and complains of lassitude. The sclerae slightly jaundiced. Tongue moist, clean, but not sore. Pulse 80, regular. Temp. 37.2. Resp. unembarrassed. Blood press. 110/50. Apex beat not definitely palpable. Left border of the heart 10 cm. from the middle line. Total cardiac diam. 13 cm. Heart sounds clear. The margin of the liver palpable below the costal arch and in the epigastrium. Slight oedema of the legs below the knees. Urine: (1/10 dilut.) Schlesinger +, trace of albumin, no pus, guaiac ÷, Bene-ict +, Gerhard +, Rothera ÷, Wassermann ÷. Serum colour 18 (21—10: 8.5). S.R. (after 1 hr.) 55 mm. (23—10—39.). Hb. 60 % = 8.28 g. %. Hb. pr. erythr. 33.1 γγ.

Erythr. 2.5 mill. Retics. 41 ‰. Leucocytes 4600. Blood smear: eos. 0.5 %, myelocytes 0.5 %, metamyelocytes 0.5 %, band forms 7 %, polymorphs 54 %, monocytes 3 %, lymphocytes 34 %, plasma cells 0.5 %, erythroblasts 1/200 L. Cabot's rings, erythroblasts with remains of nuclei, polychromasia, basophil punctuation. Marked anisocytosis, microcytosis, schizocytosis, macrocytosis. Slight pathological granulation of the leucocytes, no over-segmentation of diagnostic significance.

*Sternal puncture:* megalomacroblastosis.

A radiological examination of the heart showed nothing pathological, but it showed gastritis and a duodenal diverticulum; conditions otherwise normal.



Case 17. Cell count and Hmglb. from ear and sternal blood.

1936	Reticulocytes per mille		Hmgb. per erythrocyte in %		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
17-10	41	39	37.6	38.6	61.—	70.—	2.37	2.52	4100	64800
19-10	34	36	38.1	43.8	61.—	58.5	2.32	1.84	4300	117500
20-10	39	40	39.4	37.4	68.—	58.5	2.38	2.16	5200	65100
21-10	38	42	41.5	39.6	71.—	69.—	2.36	2.43	3800	31100
22-10	62	68	40.9	41.3	68.5	68.—	2.31	2.27	4600	147400
23-10	72	93	38.9	42.—	72.5	72.5	2.57	2.38	5100	42500
24-10	104	110	37.1	45.3	77.5	69.—	2.88	2.10	5600	93900
25-10	52									
26-10	36		38.1		76.—		2.73		5000	
27-10	20	21	37.5	40.0	76.5	67.—	2.80	2.31	4900	68600
28-10	14									
29-10	32	41	39.—	42.3	78.—	77.—	2.76	2.51	6200	62500
30-10	21									
31-10	24	61	38.4	38.2	72.—	73.—	2.59	2.66	6700	87600
1-11	29									
2-11	28		38.6		77.—		2.75		7900	
3-11	12	5	35.1	35.1	83.—	78.—	3.26	3.07	6200	31300
5-11	3		37.2		78.—		2.91		5000	
7-11	5		35.3		78.—		3.05		4100	
9-11	11		32.9		78.—		3.27		6500	
10-11	9	9	35.9	37.6	86.—	75.—	3.31	2.75	4500	30100
11-11	14									
12-11	15		35.4		81.—		3.27		7000	
13-11	11									
14-11	14		33.—		82.—		3.34		5600	
15-11	3	--								
17-11	4		30.4		82.—		3.72		5800	
20-11			28.2		83.—		4.06		5700	
23-11			29.3		85.—		4.00		5100	
26-11			29.9		83.—		3.83		6400	
27-11	1	1	30.6	33.5	84.—	80.—	3.79	3.30	3900	12800
28-11			30.—		85.—		3.91		6600	

*Treatment:* 20-10-36 4 cc. Mrk. E<sup>b</sup> (9-9) = 200 g. liver.  
 27-10-36 8 cc. Mrk. E<sup>b</sup> (9-9) = 200 g. liver.  
 10-11-36 8 cc. Mrk. E<sup>b</sup> (9-9) = 400 g. liver.  
 23-11-36 10 cc. Mrk. E<sup>b</sup> non-coloured = 500 g. liver.  
 27-11-36 10 cc. Mrk. E<sup>b</sup> non-coloured = 500 g. liver.  
 28-11-36 10 cc. Mrk. E<sup>b</sup> non-coloured = 500 g. liver.





Case 18. W. K. ♀ aged 66. Born 28—5—1870, Oslo. (Ref. nr. 433/36—37).

Occupation: Housewife.

In 1929 admitted to Ullevaal Hospital and treated for pernicious anaemia. For several years she had suffered from tiredness, lassitude and growing pallor. Tongue at times sore and red. Skin definitely yellow. Re-admitted to Ullevaal Hospital in 1931 and given liver treatment. From 1932 troubled by tingling in her feet which felt numb, so that her legs gave under her. Numbness and loss of sensation also in her hands. Admitted to the Med. Dept. A. of the Rikshospital 27—3—34 and given liver treatment as she had neglected her diet this time also. Wassermann now ÷. She felt well after discharge, but since then she has not eaten much liver, and she was re-admitted 15—7—36.

*Present condition:* She is fat, her skin a yellow white. Temp. 37.5, blood press. 140/100, pulse 72, regular. Her pupils react to light and accommodation. Tongue pale, smooth, moist. Heart 4 rib to left of sternum. Apex beat in 5th intercostal space. Blowing systolic murmur loudest over apex. Liver and spleen not palpable. Definite oedema of the lower limbs which are tender on pressure and show several extravasations of blood. The arms also are tender on pressure, showing several extravasations of blood.

<i>Reflexes:</i>	R.	L.
Biceps reflexes	÷	÷
Triceps »	÷	÷
Rad. »	÷	÷
Abdom. »	÷	÷
Patel. »	÷	÷
Achil. »	÷	÷
Plantar »	↓↓	↓↓

*Urine:* Schlesinger (1/10 dilut.) +. S.R. 38 mm. after 1 hr. Serum colour 7. Retics. 49 ‰. Hb. 38.5 % = 5.31 g. Hb. pr. erythr. 38.8 γγ. Erythrocytes 1.37 mill. Leucocytes 5200. Blood smear: bas. 0 %, eos. 0 %, metamyelocytes 0.5 %, band forms 3.5 %, polymorphs 50.5 %, monocytes 2 %, lymphocytes 43 %, erythroblasts 3/200 L. Marked poikilocytosis, schizocytosis, microcytosis, anisocytosis, macrocytosis and megalocytosis, slight anisochromia, polychromasia 1 ‰, macrothrombocytes. Over-segmentation of the nuclei of the neutrophil leucocytes.

*Sternal puncture:* Macro-megalo-blastosis.

*Treatment:* 15—7—36 18 cc. Mix.

28—7—36 15 cc. »E<sup>1</sup>»

8—8—36 20 cc. Pernami

9—8—36 20 cc. Pernami.

After her discharge she began at once to diminish the dosage of liver, and she discontinued it completely after some time. She was re-admitted 29—9—37 in about the same condition as on 15—7—36. Liver and spleen not palpable. Slight oedema of her ankles. S. R. 24 mm. Serum colour 6. Temp. 37.7.

Case 18. Differential leucocyte count of ear blood.

1936		15—7	18—7	20—7	25—8	37—9—37
Bas.	per cent .....	—	—	—	—	—
Eos.	» .....	+	1.5	0.5	2.0	1.7
Neutro- phils	young forms » .....	0.5	—	—	—	—
	band forms » .....	3.5	1.0	1.5	1.0	3.4
	polymorphs » .....	50.5	29.5	44.0	39.5	33.6
Monocytes	» .....	2.0	2.0	3.5	4.0	0.8
Lymphocytes	» .....	43.5	66.0	50.5	53.5	60.5
Macrobl.	bas. in 200 leucocytes	1	—	—	—	—
	eos. »	1	2	—	—	—
Erythrobl.	bas. »	1	—	—	—	3
	eos. »	—	2	2	—	3

Case 18. Cell count and Hmglob. from ear and sternal blood.

1936—1937	Reticulocytes per mille		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
15—7—36	49	60	38.5	37.—	1.37	1.09	5200	64300
16—7	40							
17—7	43		37.—		1.34		3500	
18—7	45	142	41.—	42.5	1.33	1.34	6500	83500
19—7	47							
20—7	35		43.		1.47		4900	
21—7	54							
22—7	59		47.—		1.52		5600	
23—7	53							
24—7	15		45. —		1.50		5600	
29—7	8		42.—		1.41		5100	
5—9	3		90.—		4.19		7400	
1—10—37	10	20	37.—	36.—	1.11	0.96	2900	226300
7—10	222							
19—10	5		53.—		1.91		6100	

Case 18. Differential cell count of sternal blood.

1936—39		15—7—36	18—7—36	1—10—37	
Sternal puncture		Asp. easy 1.1 cc. st. blood m.p. ++ f.p. 0 severe a. pain	Asp. easy 0.35 cc. st. blood m.p. ++ f.p. 0 severe a. pain	Asp. easy 0.7 cc. st. blood m.p. ++ f.p. 0 severe a. pain	
Erythropoiesis	Erythroblasts	per cent	40.4	52.—	55.2
	Leuco- blasts	»			
	cytes	»	59.6	48.—	44.8
	Promegalomacroblasts	»	11.—	1.3	11.—
	Megalobl. bas.	»	3.4	8.—	5.—
	eos.	»	3.—	1.3	—
	Macrobl. bas.	»	22.8	43.—	59.—
	eos.	»	10.4	4.3	18.—
	Normobl. bas.	»	—	9.4	—
	eos.	»	1.—	6.—	—
	Erythrobl. bas.	»	17.8	8.7	1.—
	eos.	»	29.6	17.3	5.5
	Erythrobl. bas.	»	—	0.7	0.5
division forms	eos.	1.—	—	—	
Leucopoiesis	Megacaryocytes	»	—	0.3	0.2
	Mast cells immature	»	0.25	0.7	—
	mature	»	—	—	—
	Eos. myelocytes	»	1.25	1.7	2.7
	leucocytes band forms	»	0.75	1.—	1.8
	polymorphs	»	1.—	1.—	2.5
	Myeloblasts	»	1.50	0.7	2.3
	Praemyelocytes	»	4.25	5.3	7.7
	Neutro- myelocytes	»	17.—	10.7	14.5
	philes young forms	»	26.75	25.7	38.—
	band forms	»	15.25	12.—	7.7
	polymorphs	»	11.75	13.3	4.8
	Mono- blasts	»	—	1.7	—
	cytes	»	—	—	—
	Lymphocytes	»	9.75	10.3	6.1
	Plasma & Türk cells	»	0.75	—	—
	Reticulo endothelial cells	»	+	0.3	1.1
Smear cells	»	9.75	15.3	12.6	

Case 19. G. M. R. ♂ aged 66. Born 14—12—1869, Oslo. (Ref. nr. 6475/35—36).

Occupation: Parish clerk.

Of his five brothers and sisters, four had died, one of diabetes mellitus. In 1925 he began to be troubled by bouts of diarrhoea and abdominal pain with loss of appetite. A doctor found achylia, and HCL was prescribed. Well until the autumn of 1935 when he again suffered from bouts of diarrhoea. In the summer of 1934 he began to be breathless, and a couple of months before Christmas 1935 he began to experience lassitude and tiredness, being breathless on walking up stairs and up hill. Gradually he became breathless even when walking on the flat, Latterly his skin has begun to be slightly yellow, at the same time he was troubled by giddiness and headache. In the course of about nine months he lost 14 ½ kg. (from 101 to 86.5 kg.). He was admitted to the Med. Dept. A. of the Rikshospital 10—2—1936.

*Present condition:* Face and sclerae subicteric. Afebrile. Blood press. 130/75. A distinct systolic murmur loudest over the aorta and apex. Normal findings otherwise. Ewald test meal: (3/4 hr.) ac. 0/10, Congo ÷ McLean ÷. Urine: Schlesinger (1/10 dilut.) ++. Wassermann ÷. Serum colour 6. S.R. (after 1 hr.) 61 mm. Hb. 64 % = 8.83 g. %. Hb. pr. erythr. 44.5 γγ. Erythr. 1.96 mill. Leucocytes 4200. Retics. 18 0/00. Blood smear: bas. 1 %, eos. 3 %, band forms 1.5 %, polymorphs 45 %, monocytes 2 %, lymphocytes 48.5 %. Marked megalocytosis, anisocytosis, poikilocytosis, schizocytosis, and microcytosis. Marked over-segmentation of the nuclei of the neutrophil leucocytes.

*Sternal puncture:* megalomacro-blastosis.

A radiological examination of the stomach and duodenum showed normal conditions apart from broad folds of the mucosa in the canalis portion.

Case 19. Cell count and Hmglb. from ear and sternal blood.

1936	Reticuloocytes per mille		Hmglb. pr. erythro. in γγ		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
13—2	18	29	45.—	38.4	64	49.5	1.96	1.77	5400	191900
15—2	32	43	48.6	45.9	63	57.5	1.79	1.73	6100	125400
17—2	48	84	46.5	38.8	62	63.—	1.84	1.48	5400	96700
19—2	60	54	42.5	47.5	65	56.5	2.11	1.64	5800	95200
21—2	66	66	44.5	39.4	71	62.—	2.20	2.17	5400	80600
25—2	12		43.5		64		2.03			
2—3	136									
10—3	17	18	42.9	41.1	82	77.5	2.64	2.60	6400	47200
2—4	6		38.2		81		2.93		6900	
6—4	32		34.9		94		3.43		8600	

Case 19. Differential cell count of sternal blood.

1936		13—2	15—2	17—2	19—2	21—2	10—3
Erythropoiesis	Erythroblasts per cent	35.—	48.2	39.—	37.4	25.2	12.2
	Leuco- blasts »	65.—	51.8	61.—	63.6	71.8	87.8
	cytes »						
	Promegalomacroblasts »	9.—	—	—	1.1	0.8	—
	Megalobl. bas. »	10.4	0.7	2.6	2.7	11.1	9.8
	cos. »	1.7	—	3.6	—	4.—	3.3
	Macrobl. bas. »	33.2	6.9	22.5	15.6	30.9	23.—
	cos. »	—	0.7	6.7	—	—	—
	Normobl. bas. »	—	70.6	23.6	24.3	17.5	13.1
	cos. »	0.5	21.1	41.—	15.6	9.5	19.7
	Erythrobl. bas. »	45.2	—	—	26.5	13.5	21.3
	eos. »	—	—	—	13.—	8.7	9.8
	Erythrobl. bas. »	—	—	—	2.2	4.—	—
	division forms eos. »	—	—	—	—	—	—
Leucopolesis	Megacaryocytes »	—	—	—	—	—	—
	Mast cells immature »	—	—	—	—	—	—
	mature »	—	—	0.75	—	—	—
	Eos. myelocytes »	2.5	1.50	1.50	1.00	0.75	0.8
	leucocytes band forms »	2.75	1.50	1.75	2.00	3.25	0.6
	polymorphs »	3.—	2.—	2.50	2.50	1.75	1.8
	Mycloblasts »	1.25	0.75	0.50	0.25	2.25	0.8
	Praemyelocytes »	1.75	12.50	3.50	2.—	4.75	1.2
	Neutro- myelocytes »	15.75	11.25	8.25	5.50	10.50	10.4
	philes young forms »	18.75	35.25	16.—	18.25	17.50	17.6
	band forms »	40.—	29.75	36.50	37.25	32.—	20.—
	polymorphs »	11.—	3.75	22.25	27.75	17.25	29.6
	Mono- blasts »	}	0.50	0.75	—	0.25	1.2
	cytes »						
	Lymphocytes »	1.5	0.25	3.75	1.00	6.25	15.4
	Plasma & Türk cells »	7.5	0.25	0.75	0.75	0.75	0.4
	Reticulo endothel. cells »	2.—	0.75	1.25	1.75	2.50	0.2
	Smear cells »	—	—	—	—	—	—

*Treatment:* 13—2—36 4 cc. B.F.B—P—Ph—sol = 200 g. liver.  
 25—2—36 36 cc. B.F.B—P—Ph—sol = 1800 g. liver.  
 16—3—36 6 cc. Mrk. E.B. = 300 g. liver.  
 24—3—36 36 cc. Mrk. E.B. = 1800 g. liver.  
 30—3—36 40 cc. Pernami.

Case 20. A. B. ♀ aged 74. Born 24—9—1862, Sandefjord. (Ref. nr. 4786/36—37).

Increasing lassitude during the last 3 or 4 years, and for several years breathless on walking up stairs and up hill. Never troubled by disturbances of the digestion, but has occasionally noticed soreness of the tip of her tongue. Has been troubled for some time by pain in the tips of her fingers and toes. Drafted from the Medical Polyclinic to the Med. Dept. A. of the Rikshospital 8—12—1936.

*Present condition:* She is pale and her mucous membranes are pale. State of general nutrition moderate. Temp. 37.2. Tongue slightly atrophic. Resp. unembarrassed. Blood press. 160/100. Slight oedema of the legs be-

Case 20. Differential leucocyte cell count of ear blood.

1936		9—12	12—12	16—12	17—12	31—12
Bas.	per cent . . . . .	0.5	0.5	1.0	0.5	1.0
Eos.	" . . . . .	1.0	0.5	2.5	2.0	2.0
Neutrophils	young forms "	0.5	1.5	3.0	1.0	1.5
	band forms "	3.0	8.5	5.0	7.0	7.5
	polymorphs "	57.0	38.5	42.5	40.5	38.5
Monocytes	" . . . . .	7.0	6.0	7.0	6.5	9.0
Lymphocytes	" . . . . .	31.0	47.5	39.0	42.5	40.5

Case 20. Cell count and Hinglb. from ear and sternal blood.

1936	Reticulocytes per mille		Hinglb. per erythrocyte in %		Hinglb.		Erythrocytes in millions		Ducleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
12--12	15	18	36.4	39.1	83	75	3.15	2.64	9700	30900
13--12	10									
14--12	14				79		2.82		6800	
15--12	15									
16--12	13		36.7		82		3.10		6400	
17--12	32	55	39.8	38.1	91	84	3.15	2.96	5200	59000
18--12	38									
19--12	22									
20--12	16									
21--12	16	17	36.2	46.2	85	85	3.24	2.54	5700	15400
22--12	11									
23--12	10									
24--12	8		35.4		90		3.51		6400	
1937										
5--1			31.1		90		3.99		7900	

Case 20. Differential cell count of sternal blood.

1936			12—12	17—12	21—12	
Sternal puncture			Asp. easy 0.9 cc. st. blood m.p. + f.p. +++ severe a. pain	Nsp. easy 0.65 cc. st. blood m.p. ++ f.p. +++ severe a. pain	Nsp. under vac. [20 cc.] 0.1 cc. st. blood m.p. + f.p. traces severe a. pain	
Erythropoiesis	Erythroblasts	per cent	16.--	42.2	19.5	
	Leuco-	blasts	»			
		cytes	»	84.--	57.8	89.5
	Promegalomacrobldsts	»	4.--	0.6	—	
	Megalobl.	bas.	»	5;--	0.3	--
		eos.	»	—	—	--
	Macrobl.	bas.	»	57.--	11.7	5.--
		eos.	»	14.--	2.4	2.--
	Normobl.	bas.	»	1.--	67.7	65.--
		eos.	»	12.--	16.3	20.--
Leucopoiesis	Erythrobl.	bas.	»	—	0.3	7.--
		eos.	»	5.--	—	--
	Erythrobl.	bas.	»	2.--	0.7	1.--
		eos.	»	—	—	--
	division forms	eos.	»	—	—	--
	Megacaryocytes	»	--	0.25	--	
	Mast cells	immature	»	0.25	0.50	0.25
		mature	»	0.50	--	0.50
	Eos.	myelocytes	»	1.75	1.75	0.75
	leucocytes	band forms	»	1.50	1.--	1.25
		polymorphs	»	2.50	1.50	2.25
	Myeloblasts	»	3.75	2.--	1.25	
	Praemyelocytes	»	3.75	2.50	4.--	
	Neutro-	myelocytes	»	15.25	21.75	12.75
	philes	young forms	»	27.--	28.--	18.--
		band forms	»	4.25	8.75	17.50
		polymorphs	»	28.25	14.--	24.75
	Mono-	blasts	»	1.50	1.25	2.25
		cytes	»			
	Lymphocytes	»	2.75	4.75	3.75	
	Plasma & Türk cells	»	0.25	0.25	0.25	
	Reticulo endothelial cells	»	—	—	0.25	
	Smear-cells	»	6.25	11.75	10.--	
	Unclassified cells	»	0.50		0.25	

low the knees. A normal electrocardiogram. Ewald test meal: (3/4 hr.) Congo  $\div$ , McLean  $\div$ , ac. 0/1. Urine: Schlesinger (1/10 dilut.) faint +. Wassermann  $\div$ . Serum colour 8. S.R. (after 1 hr.) 18 mm. Retics. 14 ‰. Hb. 78 % = 10.764 g. %. Hb. pr. erythr. 38.4 ‰. Erythrocytes 2.8 mill. Leucocytes 4600. Blood smear: eos. 1 %, bas. 0.5 %, metamyelocytes 0.5 %, band forms 3 %, polymorphs 57 %, monocytes 7 %, lymphocytes 31 %. Marked anisocytosis, macrocytosis, a microcyte here and there, basophil punctuation. Signs of over-segmentation of the nuclei of the neutrophil cells. Faint polychromasia.

*Sternal puncture*: megalomacroblastosis.

A radiological examination showed a moderate degree of swelling of the gastric mucosa. Stomach and duodenum otherwise normal.

*Treatment*: 12—12—36 7.5 cc. Mrk. (x)<sup>E</sup> = 11 mg.

21—12	}	60 cc. Pernami
22—12—36		
23—12		
31—12—36	}	60 cc. Pernami
1—1—37		
2—1—37		

*Case 21. A. K. ♂ aged 54. Born 20—10—1882, Sandefjord. (Ref. nr. 6069/36—37).*

*Occupation*: Ship's engineer.

«Climatic fever» (Gulf of Mexico) in 1907, gonorrhoea in 1916, ulcer molle in 1917, malaria in 1918, and black-water fever (Cape Town) in 1919. Has worked on shore since 1932. Of late years he has noticed a tendency to breathlessness and palpitation of the heart, and recently his fingers have trembled. In the late autumn of 1936, his strength and working capacity began to diminish, he had often to rest, and he suffered from weakness and lassitude. He has lost initiative and is reluctant to take on work. Can eat any kind of food, appetite excellent, his natural functions in good order. He consulted a doctor 20—1—1937, and, pernicious anaemia being diagnosed, he was admitted to the Med. Dept. A. of the Rikshospital 25—1—1937.

*Present condition*: Powers of comprehension poor, his answers lack precision, and he is rather sluggish. Easily out of breath. Pulse 88, regular. Resp. 24, audible. Temp. 38.2. Tongue smooth, its surface atrophic and marked by small furrows, moist, clean. Blood press. 140/90. Pupils equal, reacting promptly to light and accommodation. Sclerae slightly subicteric. Fine crepitation over both pulmonary bases behind. Apex beat of the heart in the 5th intercostal space, its sounds clear. Liver dulness from 6th rib to costal arch. The border of the liver just palpable. Spleen not palpable. Tremor of his hands. Reflexes normal. Ewald test meal (3/4 hr.) ac. 0/3, Congo  $\div$ , McLean  $\div$ . Wassermann  $\div$ . S.R. 9 mm. after 1 hr. Urine: Schlesinger



Case 21. Differential leucocyte count of ear blood.

1937			26—1	27—1	10—1	2—2	4—2
Bas.	»	.....	—	—	—	1.0	0.5
Eos.	»	.....	2.0	—	0.5	0.5	—
Neutrophils	myelocytes	»	—	—	—	1.0	—
	young forms	»	1.0	4.0	1.0	1.5	1.5
	band forms	»	2.0	10.0	6.0	10.5	9.0
	polymorphs	»	45.0	68.5	44.5	51.0	46.0
Monocytes	»	.....	2.0	2.5	4.5	10.0	7.5
Lymphocytes	»	.....	48.0	15.0	43.5	24.5	35.5

Case 21. Cell count and Hmglob. from ear and sternal blood.

1937	Reticulocytes per mille		Hmglob. per erythrocyte in %		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
27—1	31	22	40.4	45.1	84	88	2.87	2.69	7900	42950
28—1	33									
29—1	25									
30—1	23	40	41.8	43.7	90	92	2.92	2.93	4600	310500
31—1	41									
1—2	57									
2—2	88	88	43.3	48.6	92	87	2.93	2.47	7100	77700
3—2	74									
4—2	14	10	38.2	43.3	92	89	3.32	2.84	8100	23500
5—2	11									
6—2	7		38.1		93		3.37		5000	
9—2	2		39.5		95		3.32		6000	
18—2	25		34.6		94		3.75		7100	

(1/10 dilut.) +. Serum colour 13. Hb. 80 % = 11.04 g. %. Hb. pr. erythr. 42.5 % Erythr. 2.6 mill. Leucocytes 5000. Retics. 12 %<sub>100</sub>. Blood smear: eos. 2 %, metamyelocytes 1 %, band forms 2 %, polymorphs 45 %, monocytes 2 %, lymphocytes 48 %. Anisocytosis, basophil punctuation, microcytosis, schizocytosis, poikilocytosis, macrocytosis, polychromasia, orthochromasia, over-segmentation of the nuclei of the neutrophil leucocytes.

*Sternal puncture:* megalomacroblastosis.

*Treatment:* 27—1—1937 20 cc. E<sup>b</sup> Ph. u. praecip. Bentz. A. = 4000 g. liver.

12/16—2—1934 100 cc. Pernami.

Case 21. Differential cell count of sternal blood.

1937				27—1	30—1	2—2	4—2
Sternal puncture				Asp. easy 0.55 cc. st. blood m.p. + + f.p. + no a. pain	Asp. easy 0.6 cc. st. blood m.p. + + + + f.p. 0 moderate a. pain	Asp. easy 0.75 cc. st. blood m.p. + + f.p. + + f.p. 0 severe a. pain	Asp. easy; 0.8 cc. st. blood m.p. + f.p. 0 moderate a. pain
Erythropoiesis	Erythroblasts	per cent		23.9	52.8	44.4	25.—
	Leuco- blasts	»					
	cytes	»		76.1	47.2	55.6	7.5
	Promegalomaeroblasts	»		4.—	0.75	—	—
	Megalobl. bas.	»		2.—	—	—	—
	eos.	»		1.—	—	—	—
	Macrobl. bas.	»		51.—	13.75	22.7	25.—
	eos.	»		7.—	—	5.6	2.—
	Normobl. bas.	»		17.—	82.—	19.—	30.—
	eos.	»		8.—	1.50	50.7	34.—
	Erythrobl. bas.	»		7.—	1.—	—	1.—
	eos.	»		3.—	—	1.7	4.—
Leucopoiesis	Erythrobl. bas.	»		—	0.50	0.3	1.—
	division forms eos.	»		—	—	—	3.—
	Megacaryocytes	»		—	—	—	—
	Mast cells immature	»		0.25	0.25	0.25	0.50
	mature	»		—	—	—	0.25
	Eos. myelocytes	»		0.75	1.75	0.75	1.—
	leucoocytes band forms	»		0.75	0.75	1.25	1.50
	polymorphs	»		1.—	0.50	0.75	0.75
	Myeloblasts	»		2.—	1.25	1.25	0.25
	Praemyelocytes	»		2.—	1.25	2.75	2.25
	Neutro- myelocytes	»		11.75	29.50	13.75	15.25
	philes young forms	»		18.50	25.25	26.50	32.75
	band forms	»		12.50	14.75	12.75	15.25
	polymorphs	»		30.50	13.25	17.50	17.75
	Mono- blasts	»		0.50	0.25	—	1.75
	cytes	»		11.50	4.—	9.—	6.—
	Lymphocytes	»		0.50	0.75	—	0.25
	Plasma & Türk cells	»		—	—	—	0.25
	Reticulo endothelial cells	»		—	—	—	0.25
	Smear cells	»		7.50	6.50	13.50	4.75

Case 22. A. S. ♀ aged 17. Born 22—10—1891, Skedsmo. (Ref. nr. 979/37—38).

Treated in the Med. Dept. A of the Rikshospital for pernicious anaemia and goitre. She had been treated in the Surg. Dept. A of the same hospital between Nov. 6 and Dec. 11, 1930 for toxic goitre. A brother has been operated on for goitre. She was well till the spring of 1930 when she began to be troubled by growing nervousness. She became restless, slept badly, suffered from palpitation of the heart and swelling of her legs in the evening. Loss of weight in spite of good appetite. The Surg. Dept. A reported 6—11—1930 that she was thin, pale, restless and nervous, showing slight exophthalmus and fine tremor of her hands. Her goitre measured 5 by 10 cm. Systolic murmur over the apex. 27—11—1930 subtotal resection of the goitre. Basal metabolism 8—11—1930 150 %, 8—12—30 108 %, 2—3—1932 97 %. Was well after the operation till the autumn of 1933 when she felt relaxed and tired, becoming thin and pale. Appetite good, no dyspepsia.

Present condition: Med. Dept. A 21—8—1934 yellow, pale, relaxed and apathetic. Blood press. 180/90. Tongue clean, smooth. Apex beat heaving powerfully. A systolic murmur and a diastolic whistling murmur. Erythrocytes 1.67 mill. Leucocytes 5000. Hb. 32 %. Retic. 0.5<sup>o</sup>/<sub>100</sub>. Wassermann ÷. Electrocardiogram 29—8—1934 showed myopathia. She was treated with Pernami and was discharged as cured 5—11—34. After her discharge she continued to feel well till a fortnight before readmission. All this time she had eaten 1 kg. of liver every week (?), and once a month she had been given an injection of Pernami. A fortnight before readmission she began to suffer from palpitation of the heart and a sense of lassitude and exhaustion. She could no longer do any work, spending some of her time in bed, being breathless on walking. Her tongue had not been sore. Slept and ate well, action of the bowels, micturition and menstruation normal.

Case 22. Differential leucocyt count of ear blood.

1938		30—4	6—5
Praemyelocytes	per cent .....	—	0.5
Bas.	" .....	—	—
Eos.	" .....	0.5	3.0
Neutrophils {	myelocytes	" .....	0.5
	young forms	" .....	—
	band forms	" .....	0.5
	polymorphs	" .....	34.5
Monocytes	" .....	7.0	48.5
Lymphocytes	" .....	57.5	2.5
Erythrobl. bas.	in 200 leucocytes .....	2	—
eos.	" .....	1	1

Case 22. Cell count and Hmgb. from ear and sternal blood.

1938	Reticulocytes per mille		Hmgb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
30--4	12		35		1.11			
2--5	36							
3--5	55		37		1.26		4500	
6--5	16	23		35.5		1.19		78600
7--5	16							
8--5	10							
9--5	2		33		1.21		3100	
10--5	6	10		30.--		1.30		236200
11--5	38							
12--5	78		35		1.23		3800	
13--5	102							
14--5	125							
15--5	75							
16--5	28		35		1.20		3500	
9--6	4		84		3.93		5400	

*Present condition:* Thin and very pale with a hint of yellow. Gives the impression of great lassitude and exhaustion, drowsy. Pulse 108, regular, small. Blood press. 180/105. Resp. 24, unembarrassed. Tongue moist, clean. Temp. 37.7. Apex beat heaving in the third intercostal space. Systolic and diastolic adventitious sounds. The border of the liver palpable just below the costal arch. Spleen not palpable. June 28 basal metabolism 126 %. Wassermann ÷. Blood smear; eos. 0.5 %, myelocytes 0.5 %, polymorphs, 34.5 %, monocytes 7 %, lymphocytes 57.5 per cent. Marked anisocytosis, macro- micro-schizocytosis, poikilocytosis, polychromasia < 1 ‰, 2 bas. erythr. bl./200 L. 1 eos. erythr. bl./200 L. The neutrophil polymorph leucocytes are large and some of them show over-segmentation.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 8/5 20 cc. Pernami «Nyco».  
 9/5 20 cc. Pernami «Nyco».  
 17--18/5 40 cc. Pernami «Nyco».  
 8-- 9/6 40 cc. Pernami «Nyco».  
 28--29/6 70 cc. Pernami «Nyco».

Case 22. Differential cell count of sternal blood.

1938			6—5	9—5	10—5
Sternal puncture			Asp. easy 1.3 cc. st. blood m.p. +++++ f.p. 0 severe a. pain	Asp. under vac. [20 cc.] 0.2 cc. st. blood. The punctate coagulated	Asp. easy 0.5 cc. st. blood m.p. +++++ f.p. 0 moder- ate a. pain
Erythropoiesis	Erythroblasts	per cent	35.1	22.5	45.6
	Leuco-	blasts »			
		cytes »	64.9	77.5	54.4
	Promegalomacroblasts	»	21.—	—	6.5
	Megalobl.	bas. »	14.3	—	1.—
		cos. »	2.—	2.—	—
	Macrobl.	bas. »	52.5	52.—	80.5
		cos. »	6.—	10.—	0.5
	Normobl.	bas. »	—	—	4.5
		cos. »	—	—	—
	Erythrobl.	bas. »	1.—	16.—	3.5
		cos. »	2.5	18.—	1.5
	Erythrobl.	bas. »	0.5	2.—	2.—
	division forms	cos. »	—	—	—
Leucopoiesis	Megacaryocytes	»	0.25	—	+
	Mast cells	immature »	—	—	0.50
		mature »	—	—	—
	Eos.	myelocytes »	3.50	3.—	0.50
	leucocytes	band forms »	2.25	4.—	1.50
		polymorphs »	2.25	1.—	1.50
	Myeloblasts	»	3.75	1.—	4.75
	Praemyelocytes	»	5.75	10.—	3.—
	Neutro-	myelocytes »	15.75	10.—	10.25
	philes	young forms »	35.75	27.—	35.50
		band forms »	7.25	14.—	7.—
		polymorphs »	7.75	23.—	7.—
	Mono-	blasts »	} 1 25	—	0.25
		cytes »		—	3.75
	Lymphocytes	»	4.25	3.—	—
	Plasma & Türk cells	»	1 25	2.—	—
	Reticulo endothelial cells	»	5.—	—	4.50
	Smear cells	»	4.—	2.—	20.—

Case 23. H. T. ♂ aged 52. Born 5—1—1885, Follidal. (Ref. nr. 10875/36—37).

Occupation: Farm labourer (farmer).

Well until 1925 when he consulted a doctor for lassitude and was told his blood was thin. After treatment for some time with liver he recovered completely. He thinks he did not eat liver for more than two weeks. During the last four years he has eaten liver only occasionally when, on rare occasions, he had the opportunity to do so. During the last few weeks a sensation of slight pricking in hands and feet, a sense of lassitude and some soreness of his tongue. Appetite poor of late. He went to Oslo and consulted a doctor who secured his admission to the Med. Dept. A of the Rikshospital 23—6—1937.

*Present condition:* Thin, pale, skin yellow but not icteric. Pulse 88, regular. Mucous membranes pale. Blood press. 120/80. Tongue perfectly smooth. No enlargement of the lymphatic glands. Reflexes normal. Cardiac diam 14 cm. A faint systolic murmur over the apex. Contour of the abdomen normal. Tympanitic on percussion, no tenderness nor distension. Liver dulness from sixth rib to costal arch immediately under which the margin of the liver is palpable. Spleen palpable (?). Electrocardiogram normal.

Case 23. Cell count and Hmglob. from ear and sternal blood.

1937	Reticuloocytes per m'le		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
24—6	7		52		1.88		2700	
25—6	5							
26—6	5							
27—6	4							
28—6	7				1.54			
29—6	8							
30—6	7	13	49	47.—	1.67	1.78	2900	72900
1—7	9	16	49	41.—	1.88	1.73	4900	139500
2—7	5		48		1.54		4700	
3—7	16	51	49	39.—	1.62	1.56	4500	544300
4—7	42							
5—7	155		53		1.67		4500	
6—7	130							
7—7	111	120	54	50.5	1.94	2.11		33500
8—7	100							
9—7	91		62		2.20		6800	
10—7	72							
11—7	24							
12—7	22		62		2.16		9400	

Case 23. Cell count from ear and sternal blood.

1937			30—6	1—7	3—7	7—7
Sternal puncture			Asp. easy 0.3 cc. St. blood m. p. ++++ f. p. 0 severe a. pain	Asp. easy 0.6 cc. st. blood m.p. ++++ f.p. 0 moderate a pain	Asp. easy:0.4 cc. st. blood m.p. ++++ f.p. 0 f.p. 0 severe a. pain	Asp. easy 0.4 cc. st. blood m.p. +++ f.p. 0 moderate a pain
Erythropoiesis	Erythroblasts	per cent	32.9	43.3	65.1	29.—
	Leuco- blasts	»				
	cytes	»	67.1	56.7	44.9	71.—
	Promegalomacroblasts	»	18.5	12.5	4.5	4.—
	Megalobl. bas.	»	8.5	4.—	—	—
	eos.	»	—	—	—	—
	Macrobl. bas.	»	52.—	71.5	12.5	20.—
	eos.	»	7.—	3.5	—	2.—
	Normobl. bas.	»	—	—	77.5	35.—
	eos.	»	—	—	3.5	36.—
	Erythrobl. bas.	»	4.—	2.—	—	2.—
	eos.	»	6.5	4.5	—	1.—
	Erythrobl. bas.	»	3.5	2.—	1.5	—
	division forms eos.	»	—	—	0.5	—
Leucopoiesis	Megacaryocytes	»	+	—	—	—
	Mast cells	immature	0.75	—	—	—
		mature	0.25	—	—	—
	Eos. myelocytes	»	0.75	0.75	1.—	1.50
	leucocytes band forms	»	1.25	2.—	1.50	2.—
	polymorphs	»	0.25	1.—	0.50	2.—
	Myeloblasts	»	2.25	3.50	1.25	2.—
	Praemyelocytes	»	1.50	2.—	2.50	1.25
	Neutro- myelocytes	»	13.25	15.25	12.75	9.50
	philes band forms	»	26.50	37.50	38.75	22.75
	band forms	»	15.—	7.50	13.50	6.25
	polymorphs	»	15.75	11.—	9.50	23.—
	Mono- blasts	»	0.50	0.25	—	1.25
	cytes	»				
	Lymphocytes	»	10.25	7.75	0.50	12.25
	Plasma & Türk cells	»	0.25	—	0.25	0.50
	Reticulo endothelial cells	»	0.25	0.25	2.25	0.25
	Smear cells	»	11.25	11.25	15.75	15.50

Ewald test meal: (3/4 hr.) ac. 0/3, Congo  $\div$ , McLean  $\div$ . Urine: Schlesinger + (1/10 dilut.), alb.  $\div$ , pus  $\div$ , Benedict  $\div$ , S.R. 22 mm., Wassermann  $\div$ . Serum colour 9. Hb. 52 % = 7.176 g. %. Hb. pr. erythr. 38.2  $\gamma\gamma$ . Erythrocytes 1.88 mill. Leucocytes 2700. Retics. 7  $\%_{100}$ . Blood smear: eos. 1 %, m. myelocytes 0.5 %, band forms 3 %, polymorphs 43.5 %, monocytes 3 %, lymphocytes 49 %. Marked anisocytosis, microcytosis, schizocytosis, anisochromia, poikilocytosis, polychromasia, macro-megalcytosis. Over-segmentation of the nuclei of the neutrophil leucocytes. Basophil punctuation of the erythrocytes.

*Sternal puncture:* (30/6) megalomacroblastosis. A radiological examination of stomach and duodenum showed normal conditions.

*Treatment:* 30—6—37 4 cc. Examin »Nycos« = 200 g. liver.

*Case 24. M. S. ♂ aged 47. Born 25—11—1889, Hedrum. (Ref. nr. 10616/36—37).*

*Occupation:* Smallholder.

Well till 1927 when his stomach was resected for gastric ulcer at the Tönsberg Hospital. The last few years before this operation he had suffered from bouts of acid cructations and, two months before the operation, haemorrhage from the intestines. No troubles after the operation. In 1929 he began to scrape paint at the Navy's workshop in Sandefjord. In the summer of 1931 he consulted a doctor for lassitude and tiredness which made him fall asleep while at work. In the summer of 1932 attacks of abdominal pain for a month. A doctor diagnosed lead poisoning and the patient was put on the sick list. He lost his appetite, and in the winter of 1933 he noticed that his arms were withered, that the soles of his feet were painful, that his fingers were subject to cramp, and that he had lost weight. He was admitted to the Med. Dept. B. of the Rikshospital 7—7—1933 where he was treated for about three weeks for pernicious anaemia following gastric resection. Urobilin was found in the urine. The liver palpable two finger-breadths below the costal arch. Under liver treatment the erythrocytes rose from 2.25 to 3.96 mill. Retics. max. 140 %. At first after his discharge he was conscientious over his liver dietary, and he became fit for work in September 1933. He kept fit till the summer of 1934 when he became worse as he could not obtain liver. He improved after receiving five injections of liver from his doctor. Since then he has eaten liver except in the summer when there was little to secure. In the summer of 1935, three to four weeks would pass between each time he secured some liver of which he ate little during the following winter. Since April 1936 he has eaten no liver whatever. Two months before admission to hospital he began to be tired and weak, but he continued to work a little on his smallholding to the last. Since Oct. 1936 arms and legs have become weak and tender, and his arms have ached and felt as if they were being pricked and stabbed. Occasional cramp in arms and legs. Action of the bowels,



micturition and sleep normal. Admitted to Med. Dept. A. of the Rikshospital 15—6—1936 for anaemia.

*Present condition:* Thin and pale, mucous membranes also pale. Pulse 64, regular. Tongue smooth, furrowed. Temp. 37.4. Blood press. 160/60. No enlargement of the lymphatic glands. Reflexes and sensation normal. Cardiac diam. 13 cm., heart sounds clear. Margin of the liver not palpable. The spleen can only just be felt under the left costal arch. Wassermann ÷. No HCL found in hospital. Urine: Schlesinger ++ (1/10 dilut.), alb. ÷, pus ÷, Benedict ÷. S.R. 31 mm. Serum colour 8. Hb. 51 % = 7.038 g. %. Hb. pr. erythr. 37.4  $\gamma\gamma$ . Erythrocytes 1.88 mill. Leucocytes 3300. Retic. 20 ‰. Blood smear: Praemyelocytes 1 %, eos. 3 %, metamyelocytes 1 %, band forms 6.5 %, polymorphs 30.5 %, monocytes 5 %, lymphocytes 53 %. Marked anisocytosis, microcytosis, schizocytosis, macro-megalcytosis, poikilocytosis, anisochromia. Polychromasia 1 ‰. Basophil punctuation. Over-segmentation of the nuclei of the neutrophil leucocytes. Erythrobl. (macro) eos. 1/200 L.

*Sternal puncture:* Macro-megaloblastosis.

A radiological examination showed normal conditions apart from rapid transit of meal on account of resection of the stomach.

*Treatment:* 21—6—37 4 cc. Examin «Nyco» = 200 g. liver.

8—7—37 4 cc. Examin «Nyco» = 200 g. liver.

15—17—7—37 60 cc. Pernami.

Case 24. Differential leucocyte count of ear blood.

1937		16—6	22—6	26—6
Praemyelocytes	per cent .....	1.0	—	—
Bas.	" .....	—	1.0	—
Eos.	" .....	3.0	2.5	0.5
Neutrophils {	Myelocytes	—	—	0.5
	young forms	1.0	1.5	1.0
	band forms	6.5	9.0	5.5
	polymorphs	30.5	38.0	59.0
Monocytes	" .....	5.0	6.5	7.0
Lymphocytes	" .....	53.0	41.5	26.5
Macrobl. bas. in 200 leucocytes .....		—	—	—
	eos. " .....	1	—	—
Normobl. bas. " .....		—	—	—
	eos. " .....	—	—	—
Erythrobl. bas. " .....		—	1	—
	eos. " .....	—	1	—

Case 24. Cell count and Hmglb. from ear and sternal blood.

1937	Reticulocytes per mille		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
16-6	20	31	51	50.—	1.88	2.00	3300	55700
17-6	21							
18-6	28							
19-6	29							
20-6	28							
21-6	23	26	52	48.—	1.88	1.56	4600	79800
22-6	21	27	52	46.—	1.77	1.29	6000	69200
23-6	20							
24-6	23	48	51	55.5	1.88	1.84	7200	152800
25-6	118		52		1.77		9100	
26-6	159		53		1.97		6600	
27-6	121							
28-6	72		57		2.22		4300	
30-6	36							
1-7	12		68		2.33		5600	
2-7	7							
3-7	4		61		2.32		6000	
4-7	4							
5-7	2							
6-7	5		60		2.18		7300	
7-7	7							
8-7	5	28	62	48	2.12	2.28	6300	89500
9-7	3							
10-7	10							
11-7	6							
12-7	10	14	55	52.5	1.66	1.47	4200	83500
13-7	8							
14-7	7		60		2.14		5600	
15-7	6							
16-7	13		55		1.67		4200	

Case 24. Differential cell count of sternal blood.

1937		16—6	21—6	22—6	24—6	8—7	12—7
Sternal puncture		Asp. under vac. 0.25 cc. st. blood m.p. + + + f.p. 0 very severe a. pain	Asp. easy 0.1 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. easy 0.25 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. under vac. [20 cc.] 0.2 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. easy 0.5 cc. st. blood m.p. + + + + + f.p. 0 moderate a. pain	Asp. easy 0.4 cc. st. blood m.p. + + + f.p. traces moderate a. pain
Erythropoiesis	Erythroblasts per cent	44.1	34.2	39.3	52.9	67.8	66.7
	Leuco- blasts »	55.9	65.8	60.7	47.1	32.2	33.3
	Promegalomacroblasts »	6.5	6.—	3.5	0.5	12.—	11.—
	Megalobl. bas. »	5.—	0.5	0.5	—	8.—	3.5
	eos. »	—	—	—	—	—	—
	Macrobl. bas. »	75.—	84.5	83.5	27.—	69.—	69.5
	eos. »	9.5	3.—	3.5	5.—	2.5	0.5
	Normobl. bas. »	—	0.5	0.5	61.—	0.5	2.—
	eos. »	—	—	0.5	2.5	—	1.—
	Erythrobl. bas. »	—	0.5	4.5	0.5	2.5	7.5
	eos. »	1.—	5.—	2.—	1.5	3.—	2.—
	Erythrobl. division forms eos. »	2.—	—	1.—	2.—	2.5	3.—
Leucopoiesis	Megacaryocytes »	1.—	—	0.5	—	—	—
	Mast cells immature »	+	+	—	—	—	—
	mature »	0.25	—	1.—	1.—	—	0.25
	Eos. myelocytes »	—	—	—	—	—	—
	leucocytes band forms »	1.—	0.25	0.75	0.50	0.50	1.25
	polymorphs »	2.50	2.25	1.50	2.25	2.25	2.25
	Myeloblasts »	0.75	0.75	1.—	1.50	1.75	2.—
	Praemyelocytes »	0.50	1.25	0.75	1.25	4.25	5.25
	Neutro- myelocytes »	11.—	3.25	2.—	1.—	3.—	4.25
	philes young forms »	19.75	11.75	18.—	5.75	9.50	15.—
	band forms »	31.25	38.25	29.—	34.25	32.25	19.75
	polymorphs »	9.25	16.25	17.50	15.—	13.50	7.25
	Mono- blasts »	5.25	13.50	13.25	13.50	16.—	22.25
	cytes »	—	—	0.25	0.25	—	0.75
	Lymphocytes »	2.—	5.50	2.50	2.50	7.50	5.25
	Plasma & Türk cells »	—	—	—	—	—	—
	Reticulo endothel. cells »	—	0.50	0.25	—	0.25	0.75
	Smear cells »	16.50	6.50	12.25	21.25	9.25	13.75

Case 25. E. B. ♀ aged 66, Born 28—6—1870, Oslo. (Ref. nr. 6753/36—37).

Occupation: Widow.

Well till the autumn of 1935 when she noticed that she flagged on walking up hill and was becoming more and more tired. Of late years her tongue had occasionally been a trifle «tender». Since the beginning of February she had noticed diminished sensation in her toes which had become remarkably cold, withered and stiff on both sides. In the course of the past year she had gradually become very pale, and she had noticed that her eyes and face had become yellow. At the same time she lost much weight as measured by her clothing and not in kilos. Has not suffered from diarrhoea, giddiness or headache, and has been up and about all the time. A doctor having diagnosed anaemia 8—2—1937, she was admitted to the Med. Dept. A. of the Rikshospital.

*Present condition:* She is very pale and there is a tinge of yellow in her skin. State of general nutrition moderately good. Mucous membranes pale. Temp. 36.9. Tongue moist, clean, furrowed and smooth, showing considerable atrophy of its lining. Resp. 20, unembarrassed. Pulse 88, regular. Blood press. 115/70. Apex beat in fifth intercostal space, 9.5 cm. from the middle line. A faint systolic murmur. The margin of the liver palpable just above the transverse umbilical line. Definite oedema of the legs. Reflexes normal. Ewald test meal (after 3/4 hr.) Congo ÷, McLean ÷, ae. 0/7. Serum colour 9. Urine: Schlesinger (1/10 dilut.) +, S.R. (after 1 hr.) 22 mm. Basal metabolism 26—2—1937, 113 %, 19—3—1937, 97 %. Wassermann ÷. Retics. 9 %<sub>100</sub>. Hb. 33 % = 4.554 g. %. Hb. pr. erythr. 38.6 γγ. Erythrocytes 1.18 mill. Leucocytes 3000. Blood smear: eos. 1 %, metamyelocytes 1 % band

Case 25. Differential leucocyte count of ear blood.

1937			17—2	19—2	20—2	22—2	23—2	26—2	25—2	1—3	22—3
Praemyelocytes	»		—	—	—	—	—	—	—	0.5	—
Bas.	»		—	0.5	0.5	0.5	0.5	—	0.5	—	1.5
Eos.	»		0.5	0.5	2.5	0.5	1.5	—	0.5	3.5	6.5
Neutrophils	myelocytes	»	—	—	—	—	0.5	0.5	0.5	2.5	—
	young forms	»	7.0	—	0.5	0.5	—	—	1.0	—	0.5
	band forms	»	4.0	0.5	0.5	1.5	2.0	3.5	1.0	1.5	4.5
	polymorphs	»	43.5	45.5	59.0	53.0	39.5	47.0	42.0	40.5	68.0
Monocytes	»		—	0.5	1.0	2.0	5.5	3.0	3.0	5.5	3.0
Lymphocytes	»		50.5	52.5	30.0	42.0	50.5	46.0	51.5	46.0	16.0
Macrobl.	eos. in 200 leucocyt.		—	—	2	—	1	—	1	1	—
Normobl.	bas.	»	—	—	—	—	1	—	—	2	—
Erythrobl.	bas.	»	—	—	—	—	1	—	—	—	—
	eos.	»	1	1	—	—	—	—	2	—	—

Case 25. Cell count and Hmglob. from ear and sternal blood.

1937	Reticulocytes per mille		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
17-2	9		33		1.18		3000	
18-2	8							
19-2	6	18	31	32.5	1.01	1.00	2300	101500
20-2	18	21	31	36.—	1.01	0.96	3100	30400
21-2	8							
22-2	11	38	29	32.—	0.92	0.96	2500	96000
23-2	52	149	30	27.—	1.04	1.08	3800	131600
24-2	135	254	33	25.—	1.07	1.12	3330	52300
25-2	156	154	31	32.—	1.14	0.55	4100	174700
26-2	111							
27-2	98		31		1.01		2900	
28-2	51							
1-3	32		32		1.11		3700	
2-3	53							
3-3	352							
4-3	451	471	35	41. -	1.30	1.56	4500	89000
5-3	330							
6-3	201		40		1.67		2900	
7-3	178							
8-3	102		45		2.02		4400	
9-3	109							
10-3	125							
11-3	70		55		2.58		4300	
12-3	76							
13-3	41		56		2.51		3700	
14-3	30							
15-3	27							
16-3	34		61		2.53		4500	
17-3	21							
18-3	11		68		3.34		4900	
19-3	10							
20-3	8		70		3.47		4700	
21-3	7							
22-3	12	10	70	75.—	3.37	3.16	6000	49200
24-3	4		72		3.62		6200	
31-3			76		3.95		4700	

Case 25. Differential cell count of sternal blood.

1917		19	2	20	2	22	2	23	2	24	2	25	2	4	3	22	3
Sternal puncture		Asp. easy 0.85 cc. st. blood m.p. 4 + 4 f.p. 0 moderate a. pain		Asp. easy 0.35 cc. st. blood m.p. 4 + 4 f.p. 0 moderate a. pain		Asp. under vac. (10 cc.) 0.3 cc. st. blood m.p. 4 + 4 f.p. 0 severe a. pain		Asp. under vac. (10 cc.) 0.7 cc. st. blood m.p. 4 + 4 f.p. 0 severe a. pain		Asp. easy 0.5 cc. st. blood m.p. 4 + 4 f.p. 0 severe a. pain		Asp. easy 0.5 cc. st. blood m.p. 4 + 4 f.p. 0 severe a. pain		Asp. easy 0.5 cc. st. blood m.p. 4 + 4 f.p. 0 moderate a. pain		Asp. easy 0.55 cc. st. blood m.p. 4 + 4 f.p. 0 moderate a. pain	
Erythroblasts	per cent	37.5	39.7	53.2	13.8	39.2	40. —	58.4	23.4								
Leuco	blasts	62.5	69.3	46.8	56.2	60.8	60. —	41.6	76.6								
	cytes	4.5	2.	1.	1.25	2.	4. —	—	—								
Promyeloblasts	bas.	9.5	1. —	1.25	0.75	1.50	1.50	—	—								
Megakobl.	eos.	—	—	—	0.25	—	—	—	—								
Microbl.	bas.	58.5	67. —	15.25	36.50	52.5	73.5	15.25	10. —								
	eos.	9.	8.	5.25	5. —	4. —	6	0.50	—								
Normobl.	bas.	—	—	33. —	24.75	13.5	3. —	73.50	69. —								
	eos.	—	—	1.50	26.25	20. —	7.5	9.50	16. —								
Erythrobl.	bas.	3.	3. —	1.50	3.25	2. —	1.5	0.25	1. —								
	eos.	13. —	19. —	5.50	1.75	0.5	2. —	0.25	—								
Erythrobl.	bas.	2.	—	2.75	0.25	2. —	1.	0.75	1. —								
Division forms	eos.	0.5	—	—	—	—	—	—	—								
Megakaryocytes		4	—	0.25	4	0.50	—	—	—								
Met. cells	immature	0.50	—	0.50	0.25	0.25	0.25	0.75	1. —								
	mature	0.25	—	—	—	—	—	0.25	0.25								
Eos.	myelocytes	1.75	0.3	1. —	2.50	1.50	2. —	1. —	1.50								
Leucocytes	band forms	1.75	3. —	0.75	2.25	1.25	1. —	0.50	1. —								
	polymorphs	0.50	0.7	1. —	2. —	1.25	0.75	1.50	1.50								
Myeloblasts		6. —	2. —	3.25	3.50	4.75	3.75	3.25	2. —								
Præmyelocytes		5.75	3.7	4.50	3.50	6. —	4. —	2.75	3.50								
Neutro	myelocytes	17.25	9.3	10.25	12.25	18. —	18.75	6.50	7.50								
Philes	young forms	25.25	19.3	23. —	22. —	19. —	23.75	17.75	24.25								
	band forms	7.75	9.	7. —	9.50	7.50	11.50	8.25	12.50								
	polymorphs	6.50	13.2	9.50	5.50	9.25	5.25	18.75	22.50								
Mono-	blasts	0.50	0.7	1.75	2. —	1.25	1.75	1. —	1.75								
	cytes	13.50	25. —	16.25	20. —	12.75	9.25	19.50	12.75								
Lymphocytes		0.50	0.3	0.75	—	0.50	0.25	—	1. —								
Plasma & Türk cells		0.25	—	—	—	0.25	0.25	—	—								
Refractive endothelial cells		12.	14.4	20.25	14.75	16. —	17.50	18.25	7. —								
Streak cells		—	—	—	—	—	—	—	—								

forms 4 %, polymorphs 43.5 %, lymphocytes 50.5 %. Anisocytosis, schizocytosis, microcytosis, macro-megalocytosis, polychromasia 1 ‰. Erythroblasts 1/200 L. Over-segmentation of the nuclei of the neutrophil cells.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 19—2—37 4 cc. merk (X)<sup>c</sup> = 2 mg. t.s.  
 27—2—37 20 cc. merk (X)<sup>c</sup> = 10 mg. t.s.  
 2—3—37 10 cc. merk (X)<sup>c</sup> = 5 mg. t.s.  
 6—3—37 20 cc. merk (X)<sup>c</sup> = 10 mg. t.s.  
 11—3—37 20 cc. merk (X)<sup>c</sup> = 10 mg. t.s.  
 16—3—37 20 cc. merk (X)<sup>c</sup> = 10 mg. t.s.  
 20—3—37 20 cc. merk (X)<sup>c</sup> = 10 mg. t.s.  
 24—3—37 12 cc. merk (X)<sup>c</sup> = 6 mg. t.s.

*Case 26.* ♀ *A. B.* aged 58. Born 6—5—1878, Vestre Aker. (Ref. nr. 8990/36—37).

*Occupation:* Widow.

About Christmas 1936 she began to suffer from lassitude and tiredness. In February 1937 she came to look pale, and she was very tired early in April. She has never noticed soreness of her tongue, paraesthesias, loss of appetite or digestive disturbances. She sleeps well, but is apt to be constipated. No disturbances of micturition. Menopause five years earlier after abrasio. Treated in 1935 for myxoedema. On June 18 of this year, her basal metabolism in hospital was 79 %. She was given thyreoidin, and on August 2 her basal metabolism was 98 %. She was admitted to the Med. Dept. A. of the Rikshospital 27—4—1937.

*Present condition:* State of general nutrition very good, and she is almost plump. Very pale. No pain. Temp. 38.6. Tongue furrowed, moist, neither coated nor smooth. Blood press. 135/100. Pulse 88, regular. Resp. 16, unembarrassed. Sclerae subicteric. No enlargement of the lymphatic glands. Liver not palpable, no oedema nor rash. A suspicion of a slight systolic, blowing murmur over the apex. Reflexes normal. An electrocardiographic examination 7—5—1937 normal. Ewald test meal (3/4 hr.) ac. 0/4, Congo ÷, McLean ÷. Urine: Schlesinger (1/10 dilut.) +, alb. ÷. Wassermann ÷. S.R. 55 mm. after 1 hr. Serum colour 10. Retics. 11 ‰. Hb. 38 % = 5.244 g. %. Hb. pr. erythr. 47.3 γγ. Erythrocytes 1.13 mill. Leucocytes 4400. Blood smear: bas. 0.5 %, eos. 2.5 %, myelocytes 2 %, meta-myelocytes 1 %, band forms 8 %, polymorphs 64.5 %, monocytes 1 %, lymphocytes 20.5 %, macroblasts 1/200 L. Anisocytosis, macro-megalocytosis, microcytosis, schizocytosis, poikilocytosis, polychromasia, over-segmentation of the nuclei of the neutrophil cells.

*Sternal puncture:* Macro-megalo-blastosis.

*Treatment:* 30—4—1937 2 cc. (X)<sup>E</sup> = 1 mg. t.s. (intravenous)  
 4—5—1937 10 cc. (X)<sup>E</sup> = 5 mg. t.s.      »  
 14/18—5—1937 100 cc. Pernami.

Case 26. Cell count and Hmglb. from ear and sternal blood.

1937	Reticulocytes per mille		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
28-4			38		1.13		4400	
29-4	11	9	37	41.—	1.10	1.02	4200	129000
30-4	10	12	38	40.—	1.24	1.03	4000	88000
1-5	9	12	38	38.5	1.27	1.04	3800	29900
2-5	8							
3-5	10	10	39	40.5	1.27	0.95	5900	67800
4-5	11	14	39	36.—	1.19	0.75	4900	25800
5-5	22	22	37	52.—	1.11	1.44	5300	14000
6-5	19		37		1.09		4600	
7-5	25	28	37	39.—	1.03	0.95	6900	113200
8-5	76							
9-5	118		39		1.19		5600	
10-5	87	165	40	39.5	1.30	1.38	4600	34800
11-5	94							
12-5	79		40		1.28		4300	
13-5	49							
14-5	22		40		1.24		5000	
15-5	10							
16-5	11		40		1.33		4700	
17-5								
18-5	152		40		1.30		6100	
19-5	177							
20-5	246		43		1.75		4400	
31-5	20		60		2.54		5000	

Case 27. I. H. ♀ aged 37. Born 23—3—1899, Sweden. (Ref. nr. 5311/36—37).

Occupation: Wife of bridge-builder.

Rheumatic fever in 1918. Ten years ago, when she gave birth to her second child, she is said to have had haemoglobin 60 %. Since then she has taken iron, particularly in the winter, and she had thus been able to keep fit till the present summer when, in spite of taking iron, she began to feel more tired. She has lost weight, her skin has turned yellow, and she has been breathless on walking up stairs and up hill. For four years has been troubled at intervals by soreness of her tongue and of the inner side of both lips. On account of dyspepsia three years earlier she took a test meal said to have shown anacidity. Eight days ago, toothache-like pain in her right leg. Admitted to the Med. Dept. B. of the Rikshospital 30—12—1936.



Case 26. Differential cell count of sternal blood.

1937			29-4	30-4	1-5	3-5	4-5	5-5	7-5	10-5
Sternal puncture			Asp. easy 0.55 cc. st. blood m.p. + + + + + f.p. 0 severe a pain	Asp. easy 0.4 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. under vac. [20 cc.] 1.0 cc. st. blood m.p. + + f.p. traces moderate a. pain	Asp. easy 0.2 cc. st. blood m.p. + + + + + f.p. 0 moderate to severe a. pain	Asp. under vac. [20 cc] 0.35 cc. st. blood m.p.: traces f.p. 0 severe a. pain	Asp. under vac. [20 cc.] 0.1 cc. st. blood m.p. traces, f.p. 0 no asp. pain. Probably capillary blood mixed to	Asp. easy 0.35 cc. st. blood m.p. + + + + + f.p. 0 moderate a. pain	Asp. easy 0.2 cc. st. blood m.p. + f.p. + moderate a. pain
Erythropoiesis	Erythroblasts	per cent	38.—	33.7	33.3	43.5	29.2	10.—	53.1	33.8
	Leuco- blasts	»	62.—	66.3	66.7	56.5	70.8	90.—	46.9	66.2
	cytes	»	—	—	—	—	—	—	—	—
	Promegalomacroblasts	»	5.5	4.—	6.—	4.—	7.—	—	0.7	1.—
	Megalobl. bas.	»	11.5	5.5	7.—	8.—	7.—	—	0.3	0.5
	eos.	»	—	—	—	—	—	—	—	—
	Macrobl. bas.	»	75.—	81.—	77.5	73.—	60.—	10.—	14.—	24.—
	eos.	»	1.5	5.5	6.5	9.5	—	—	0.7	—
	Normobl. bas.	»	—	—	—	0.5	—	—	81.7	46.—
	eos.	»	—	—	0.5	—	—	10.—	1.3	23.5
Leucopoiesis	Erythrobl. bas.	»	0.5	—	0.5	0.5	7.—	40.—	—	3.—
	eos.	»	2.5	3.5	1.—	1.5	9.—	40.—	—	1.5
	Erythrobl. bas.	»	3.5	0.5	1.—	3.—	—	—	1.3	0.5
	division forms eos.	»	—	—	—	—	1.—	—	—	—
	Megacaryocytes	»	—	—	+	+	—	—	0.25	+
	Mast cells immature	»	—	0.50	0.25	0.25	0.75	—	—	1.25
	mature	»	—	—	—	0.25	—	—	—	—
	Eos. myelocytes	»	1.75	1.—	0.75	1.50	0.75	1.—	0.75	1.—
	leucocytes band forms	»	2.75	1.75	2.75	1.25	1.75	—	2.—	1.25
	polymorphs	»	1.25	1.—	2.50	0.25	2.50	—	0.75	1.25
	Myeloblasts	»	1.25	1.—	2.—	2.—	0.50	—	1.75	1.—
	Praemyelocytes	»	3.25	0.75	2.—	1.75	1.50	6.—	4.50	2.—
	Neutro- myelocytes	»	19.50	11.75	9.50	12.25	10.—	9.—	17.—	11.25
	philes young forms	»	29.—	34.75	24.50	27.25	23.25	8.—	27.—	25.—
	band forms	»	12.25	17.25	10.50	16.50	16.25	14.—	9.—	13.75
	polymorphs	»	11.75	10.25	17.50	13.50	20.—	34.—	9.75	15.75
	Mono- blasts	»	—	—	0.75	—	—	—	—	1.—
	cytes	»	—	—	—	—	—	—	—	—
	Lymphocytes	»	2.75	5.75	12.—	5.25	11.75	11.—	3.25	13.—
	Plasma & Türk cells	»	—	0.50	—	—	—	—	—	—
	Reticulo endothelial cells	»	1.—	—	0.25	0.25	0.75	—	—	—
Smear cells			13.50	13.75	15.—	17.75	10.25	17.—	24.—	13.—

Case 27. Differential leucocyte count of ear blood.

1936—1937		31—12	4—1	16—1	22—1
Pas.	per cent. ....	—	0.5	0.5	—
Eos.	» .....	2.5	3.0	4.0	1.0
Neutrophils	myelocytes	—	1.5	—	—
	young forms	—	2.0	—	—
	band forms	4.5	3.5	0.5	1.5
	polymorphs	45.0	39.5	50.0	60.0
Monocytes	» .....	4.5	5.0	3.5	6.0
Lymphocytes	» .....	43.0	45.0	41.5	31.5
Plasma-Türk	» .....	0.5	—	—	—

Case 27. Cell count and Hmglob. from ear and sternal blood.

1936 1937	Reticulocytes per mille		Hmglob. per erythrocyte in %		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
31—12	—	2	36.7		42		1.58		2300	
1—1	1									
2—1	2		36.3		45		1.71		3600	
3—1	3									
4—1	136	231	35.7	33.7	51	45	1.97	1.84	4300	370100
5—1	210									
6—1	221		37.3		54		2.00		3400	
7—1	59									
8—1	20		39.1		55		1.94		3700	
9—1	22									
11—1	13	12	36.3	37.8	61	62	2.32	2.26	6400	119300
16—1			38.6		63		2.25		5600	
22—1			36.3		72		2.74		6500	

Present condition: Skin a strikingly waxy yellow colour, the mucous membranes very pale. Pulse 80, regular. Resp. 20, unembarrassed. Temp. 37.5. Blood press. 120/65. Margins of the tongue slightly sore. Some atrophy of its papillae. Slight soreness of the inner side of the lips, and some rubor of their mucous membranes. Pupils equal, reacting to light and accommodation. Cardiac dulness 4 C. to left of sternal margin. Apex beat in fifth intercostal space in the midclavicular line. Over the whole of her heart there is a systolic blowing murmur loudest over the apex. Liver dulness from sixth rib to 3 finger-breadths below the costal margin where the border of the liver is palpable. (The liver could not be felt 22—11 1936). Spleen palpable below the costal arch, but not projecting beyond it. (The

Case 27. Differential cell count of sternal blood.

1936—37			31—12	4—1	11—1
Sternal puncture				Asp. easy 0.55 cc. st. blood m.p. +++++ f. p. 0 severe a. pain	Asp. easy 0.5 cc. st. blood m.p. +++++ f. p. 0 severe a. pain
Erythropoiesis	Erythroblasts	per cent	22.—	63.—	20.5
	Leuco- blasts	»			
	cytes	»	78.—	37.—	79.5
	Promegalomacroblasts	»	6.—	0.25	4.—
	Megalobl. bas.	»	10.—	0.25	—
	eos.	»	—	—	—
	Macrobl. bas.	»	36.—	6.—	73.—
	eos.	»	25.—	0.25	5.—
	Normobl. bas.	»	—	90.—	6.—
	bas.	»	—	2.75	5.—
	Erythrobl. bas.	»	10.—	—	3.—
	eos.	»	10.—	—	1.—
	Erythrobl. bas.	»	3.—	0.50	3.—
	division forms eos.	»	—	—	—
Leucopoiesis	Megacaryocytes	»	+	—	—
	immature	»	0.25	0.25	—
	mature	»	—	—	—
	Eos. myelocytes	»	0.25	1.50	0.25
	leucocytes band forms	»	2.—	0.75	1.—
	polymorphs	»	1.75	0.25	1.25
	Myeloblasts	»	1.25	2.50	0.75
	Fraemyelocytes	»	1.50	4.—	2.75
	Neutro- myelocytes	»	10.25	24.50	18.50
	philes young forms	»	21.50	30.75	31.25
	band forms	»	14.75	11.—	11.75
	polymorphs	»	30.75	6.50	18.75
	Mono- blasts	»	1.75	0.75	0.75
	cytes	»			
	Lymphocytes	»	10.—	0.75	5.75
	Plasma & Türk cells	»	0.50	1.—	0.25
	Reticulo endothelial cells	»	—	—	—
	Smear cells	»	3.50	15.50	7.—

spleen could not be felt 22—11—1936). No enlargement of the lymphatic glands. Ewald test meal: Congo  $\div$ , ac. 0/6. Urine: Schlesinger (1/10 dilut.) +. Wassermann  $\div$ . S.R. 46 mm. after 1 hr. Serum colour 6. Hb. 42 % = 5.796 g. %. Hb. pr. erythr. 36.7  $\gamma\gamma$ . Erythrocytes 1.58 mill. Leucocytes 2300. Retics. 1 %<sub>100</sub>. Blood smear: bas. 0 %, eos. 2.5 %, band forms 4.5 %, polymorphs 45 %, monocytes 4.5 %, lymphocytes 43 %, plasma — Türk 0.5 %. Anisocytosis, schizocytosis, microcytosis. macro-megalo-cytosis, orthochromia, over-segmentation of the neutrophil leucocytes.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 31—12—36 Injection of liver preparation  
11— 1—37 Injection of liver preparation

*Case 28. H. H. ♂ aged 62. Born 19—7—1874, Moss. (Ref. nr. 6266/37).*

*Occupation:* School teacher.

Well till May, 1936, when he began gradually to become more and more relaxed, tired and weak. Loss of appetite and steady loss of weight — about 10—12 kgs. No discomfort apart from breathlessness on exertion. Late in the autumn of 1936 troubled occasionally by slight giddiness, particularly on getting out of bed. This trouble has increased greatly, and by Jan. 20, 1937 he could no longer work as a teacher in a primary school. The doctor he consulted recommended admission to hospital. Never subject to diarrhoea, being more inclined to constipation of slight degree. No soreness of the tongue. During the last few weeks he has noticed a slight tingling and pricking in the tips of his fingers and the palms of his hands. He was admitted to the Med. Dept. A. of the Rikshospital 30—1—1937.

*Present condition:* He looks ill, his complexion is greyish-white with a touch of yellow in it, and his mucous membranes are pale. Becomes breathless on speaking or moving. Temp. 37.4. Tongue pale, smooth, atrophic, clean and moist. Resp. 26, audible. Pulse 88, regular. Blood press. 150/80. Sclerae a trifle yellow. Apex beat in the fifth intercostal space 10.5 cm. from the middle line. A slight systolic murmur. The margin of the liver palpable just above the umbilical transverse line. On 8—3—1937 it was palpable one finger-breadth below the costal arch. Reflexes normal. Ewald test meal (after 3/4 hr.) Congo  $\div$ , McLean  $\div$ , ac. 0/4. Urine: Schlesinger (1/10 dilut.) + +. Wasserman  $\div$ . Serum colour 14. S. R. (after 1 hr.) 33 mm. Retics. 12 Hb. 47 % = 6.486 g. %. Hb. pr. erythr. 46.3  $\gamma\gamma$ . Erythrocytes 1.4 mill. Leucocytes 2700. Blood smear: eos. 2 %, metamyelocytes 2 %, band forms 2 %, polymorphs 40.5 %, monocytes 4 %, lymphocytes 49.5 %. Marked anisocytosis, microcytosis, schizocytosis. Orthochromia, polychromasia, macro-megalo-cytosis. Basophil punctation. Cabot's rings, No over-segmentation of the nuclei of the neutrophil cells.

*Sternal puncture:* Megalo-macroblastosis. A radiological examination of the stomach and duodenum showed canalis gastritis and a diverticulum of the duodenum, but nothing else of interest.

Case 28. Differential leucocyte count of ear blood.

1937	1-2	3-2	5-3	8-2	9-2	10-2	11-2	12-2	13-2	14-2	15-2	20-2	23-2	22-3
Myeloblasts per cent	—	—	—	—	—	0.5	—	—	—	—	—	—	—	—
Pracmyelocytes	—	1.0	—	—	0.5	0.5	1.0	—	—	—	—	—	—	—
Bas. L.	—	0.5	—	—	0.5	0.5	0.5	—	—	—	1.0	1.5	—	0.5
Eos. L.	2.0	2.5	3.5	2.0	1.0	2.0	3.0	3.0	2.0	2.0	4.0	3.5	3.0	15.5
{ myelocytes	—	—	—	—	—	—	1.0	—	0.5	1.0	0.5	—	—	—
{ young forms	2.0	—	1.0	1.5	1.5	—	1.0	0.5	0.5	1.0	—	0.5	—	1.5
{ band forms	2.0	5.0	2.5	4.5	6.0	6.0	4.5	5.5	2.5	1.5	5.0	4.5	6.5	4.5
{ polymorphs	40.5	37.5	38.0	36.0	43.5	37.0	50.5	31.5	44.5	28.5	37.0	55.5	55.5	55.5
Monocytes	4.0	0.3	4.0	1.0	3.0	3.5	2.5	6.0	5.0	5.0	9.0	3.0	5.0	2.0
Lymphocytes	49.5	43.0	51.0	54.0	55.0	50.0	36.0	53.5	45.0	61.0	43.5	31.5	30.5	20.5
Plasma-Türk	—	—	—	1.0	—	—	0.5	—	—	—	—	—	—	—
Normobl. bas. in 200	—	—	—	—	—	—	—	1	—	—	—	—	—	—
eos. leucocyt.	—	—	—	—	—	—	1	5	4	1	—	—	—	—
Macrobl. bas.	—	—	2	—	—	—	—	1	—	—	—	—	—	—
eos.	—	1	—	—	1	1	1	1	—	1	—	—	—	—
Erythrobl. bas.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
eos.	1	—	—	—	—	1	1	—	1	—	—	—	—	—

Case 28. Cell count and Hmglb. from ear and sternal blood.

1937	Reticulocytes per mille		Hmglb. pr. erythrocyte in %		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
1—2	12	15	47	43.—	1.40	1.20	2700	105900
3—2	12	21	46	45.—	1.45	1.17	1800	88300
5—2	16		48		1.51			
8—2	25	19	46	46.—	1.42	1.47	3100	41000
9—2	17	15	47	49.—	1.60	1.96	5400	25700
10—2	16	80	46	41.—	1.51	1.36	3200	136500
11—2	25	168	46	41.—	1.49	1.45	3500	341500
12—2	47	151	47	44.5	1.52	1.59	2300	39900
13—2	158	196	47	45.—	1.75	1.53	2700	63100
14—2	116						3800	
15—2	101	145	52	56.—	1.85	1.80	4000	36300
18—2	29		59		2.44		7400	
20—2	22	11	60	61.—	2.26	2.17	5000	12600
26—2	180		62		2.20		5200	
9—3	52		74		3.12		10900	
22—3	9	20	88	75.—	4.35	3.20	8400	45600

*Treatment:* 1—2—37 3 cc. E<sup>b</sup> Ph. u. praec. Bentz. A = 600 g. liver.  
 8—2—37 8 cc. merk. (X)<sup>e</sup> = 4 mg. t.s.  
 20—2—37 30 cc. merk. (X)<sup>e</sup> = 15 mg. t.s.  
 4—3—37 20 cc. merk. (X)<sup>e</sup> = 10 mg. t.s.  
 10—3—37 20 cc. merk. (X)<sup>e</sup> = 10 mg. t.s.  
 15—3—37 20 cc. merk. (X)<sup>e</sup> = 10 mg. t.s.  
 20—3—37 20 cc. merk. (X)<sup>e</sup> = 10 mg. t.s.  
 24—3—37 12 cc. merk. (X)<sup>e</sup> = 6 mg. t.s.

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*Case 29. A. P. ♂ aged 52. Born 9—5—1886. (Ref. nr. 279/39).*

*Occupation:* Brick-kiln worker.

Subject for 6 to 7 years to bouts of giddiness and vomiting of a few minutes' duration. At the end of June, 1939, he had one of these bouts during which he collapsed and was unconscious for a moment. Since then he has suffered from lassitude and giddiness and has easily become breathless. Appetite poor of late, but his food has not troubled him. He was admitted to the Med. Dept. of the Drammen Hospital on 7—7—1939, when his state of general nutrition was moderately good, and there was no oedema or rash. Mucous membranes pale. Temp. 37.2, blood press. 110/60. Slight jaundice

Case 28. Differential cell count of sternal blood.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
--	---	---	---	---	---	---	---	---	---	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	-----

1937		Sternal puncture.											
		per cent	Asp. easy 0.9 cc. st.-blood m.p. + +++ f.p. 0 severe a. pain	Asp. easy 0.65 cc. st.-blood m.p. + +++ f.p. 0 severe a. pain	Asp. easy 1.1 cc. st.-blood m.p. + f.p. + severe a. pain	Asp. easy 0.3 cc. st.-blood m.p. + f.p. 0 moderate a. pain	sp. easy 0.3 cc. st.-blood m.p. + +++ f.p. 0 severe a. pain	sp. easy 0.5 cc. st.-blood m.p. + +++ f.p. 0 severe a. pain	p. easy 0.6 cc. blood m.p. + f.p. 0 severe a. pain	p. easy 1.4 cc. blood m.p. + f.p. 0 severe a. pain	easy 0.6 cc. blood m.p. + moderate a. pain	easy 0.2 cc. blood m.p. + moderate a. pain	
Erythroblasts		59.7	61.2	40.1	53.3	76.5	76.9	70.7	61.5	37.7	7.—	19.7	
Leuco- blasts		40.3	38.8	59.9	46.7	23.1	23.1	29.3	38.5	62.3	93.—	80.3	
cytes		4.—	0.3	6.—	0.5	—	0.4	—	1.5	0.5	—	—	
Promegakaryoblasts		15.—	0.3	—	—	—	—	—	—	—	—	—	
bas.		1.—	0.3	—	—	—	—	—	—	—	—	—	
Megalobl.		44.5	71.7	78.—	77.—	18.6	18.6	18.—	19.—	22.5	12.—	10.—	
eos.		3.—	2.—	9.—	1.—	1.—	1.—	1.25	32.5	6.—	3.—	2.—	
bas.		—	—	—	—	76.2	76.2	54.75	46.—	39.—	21.—	50.—	
Macrobl.		—	—	—	—	3.—	3.—	25.75	—	1.5	23.—	36.—	
eos.		—	0.3	—	—	—	—	—	—	0.5	26.—	1.—	
bas.		—	—	—	—	—	—	—	—	—	3.—	—	
Normobl.		31.5	24.—	2.5	14.—	—	—	0.25	+	—	1.—	—	
eos.		0.5	1.—	2.5	4.5	0.8	0.8	—	+	—	—	—	
bas.		0.5	0.4	1.—	2.—	—	—	—	+	—	—	—	
Erythrobl.		—	—	—	—	—	—	—	—	—	—	—	
Erythrobl.		—	—	—	—	—	—	—	—	—	—	—	
division forms		—	—	—	—	—	—	—	—	—	—	—	
cos.		—	—	—	—	—	—	—	—	—	—	—	
Megakaryocytes		—	—	—	—	—	—	—	—	—	—	—	
Mast cells		—	—	—	—	—	—	—	—	—	—	—	
immature		—	—	—	—	—	—	—	—	—	—	—	
mature		—	—	—	—	—	—	—	—	—	—	—	
myelocytes		—	—	—	—	—	—	—	—	—	—	—	
Eos.		—	—	—	—	—	—	—	—	—	—	—	
leucocytes		—	—	—	—	—	—	—	—	—	—	—	
band forms		—	—	—	—	—	—	—	—	—	—	—	
polymorphs		—	—	—	—	—	—	—	—	—	—	—	
Myeloblasts		—	—	—	—	—	—	—	—	—	—	—	
Pracmyelocytes		—	—	—	—	—	—	—	—	—	—	—	
myelocytes		—	—	—	—	—	—	—	—	—	—	—	
Neutro-		—	—	—	—	—	—	—	—	—	—	—	
philes		—	—	—	—	—	—	—	—	—	—	—	
young forms		—	—	—	—	—	—	—	—	—	—	—	
band forms		—	—	—	—	—	—	—	—	—	—	—	
polymorphs		—	—	—	—	—	—	—	—	—	—	—	
blasts		—	—	—	—	—	—	—	—	—	—	—	
Mono-		—	—	—	—	—	—	—	—	—	—	—	
cytes		—	—	—	—	—	—	—	—	—	—	—	
Lymphocytes		—	—	—	—	—	—	—	—	—	—	—	
Plasma & Türk cells		—	—	—	—	—	—	—	—	—	—	—	
Reticulo endothelial cells		—	—	—	—	—	—	—	—	—	—	—	
Smear cells		—	—	—	—	—	—	—	—	—	—	—	

Tongue not atrophied. Liver and spleen not palpable. No enlargement of the lymphatic glands. Urine: Schlesinger (1/10) ÷. Ewald test meal: ac. 0/4. Wassermann ÷. Urea 60 mg. %. S.R. 9 mm. Osmotic resistance: incipient haemolysis at 0.48 % NaCl., and total haemolysis at 0.38 %. Blood smear from ear blood: eos. L. 1 %, polymorphs 45 %, monocytes 1 %, lymphocytes 53 %. Orthochromia, marked anisocytosis, macro-megalo-cytosis, basophil punctuation, schizo-micro-poikilocytosis. Marked over-segmentation of the neutrophil leucocytes. Macroleucocytosis.

*Sternal puncture:* Macro-megaloblastosis.

Liver extract mrk. II (8—5—39) »Nycor» 25 cc.

1939	Reticulo-cytes ‰	Hmgbl.	Leuco-cytes	Serum colour	Erythrocytes in millions	Nuc. cells in sternal blood
8.7	15	75	4200	14	2.46	240000
10.7	77					400000
11.7	104					
12.7	122	82	6900	9	2.66	240000
13.7	179					
14.7	140					
15.7	90	90	7300		3.26	
17.7	30	90			3.33	

*Case 30. M. H. ♀ aged 55. Born in Sandsvär (Buskerud). (Ref. nr 459/39).*

For several years she had suffered from vague dyspeptic symptoms, notably meteorism and nausea after practically every kind of food. Of late years she had experienced increasing lassitude, giddiness, tinnitus and slight palpitation of the heart. From time to time her eyes had been a trifle yellow, and there had been slight oedema of her legs. Her appetite had gradually waned, and she had lost weight. During the past few months she had been troubled by fleeting pains in her legs. Iron had been prescribed, but as she did not improve, she was admitted to the Med. Dept. of the Drammen Hospital 21—7—1939.

Present condition: She is thin and there is definite oedema of her legs. Over trunk, neck and forehead are numerous dark brown patches of various sizes. Mucous membranes pale and conjunctivae a trifle jaundiced. Tongue moist and smooth. Normal conditions in other respects, liver and spleen not being palpable, and the lymphatic glands showing no pathological enlargement. A neurological examination negative apart from the Achilles reflexes which cannot be evoked. Wassermann ÷. Urea 0.30 mg. %. Ewald test meal: ac. 0/3. S.R. 25 mm. Urine: Schlesinger (1/10 dilut.) +. Temp. 37.5. Blood press. 135/75. Blood smear from ear: eos. L. 2 %, band forms 4 %, polymorphs 43 %, monocytes 7 %, lymphocytes 44 %. Marked anisocytosis,



Case 29. Differential cell count of sternal blood.

1939		8—7	10—7	12—7
Sternal puncture		Asp. easy 0.9 cc. st. blood m.p. +++ f.p. 0 severe a. pain	Asp. easy 1.1 cc. st. blood m.p. +++ f.p. 0 severe a. pain	Asp. easy 0.5 cc. st. blood m.p. +++ f.p. 0 severe a. pain
Erythropoiesis	Erythroblasts per cent	61.30	73.10	48.20
	Leuko- blasts »			
	cytes »	38.70	66.90	51.80
	Pro- megalobl. »			
	makrobl. »	2.—	3.50	0.50
	Megalobl. bas. »	9.—	—	—
	eos. »	—	—	—
	Macrobl. bas. »	54.60	59.50	22.50
	eos. »	15.70	32.50	1.—
	Normobl. bas. »	2.70	3.50	37.—
	eos. »	3.30	—	38.—
	Erythrobl. bas. »	3.—	—	—
	eos. »	5.70	—	—
	Erythrobl. bas. »	3.—	0.50	1.—
	eos. »	—	—	—
	division forms eos. »	1.—	0.50	—
Leucopoiesis	Megacaryocytes »	0.50	0.25	0.25
	Mast cells immature »	0.25	1.25	0.25
	mature »	—	—	—
	Eos. myelocytes »	2.75	5.50	2.—
	leucocytes band forms »	4.25	2.—	1.50
	polymorphs »	1.25	1.75	1.75
	Myeloblasts »	5.50	4.—	2.50
	Pracmyelocytes »	5.25	9.75	7.—
	Neutro- myelocytes »	12.25	13.50	8.75
	philes young forms »	32.50	25.50	18.75
	band forms »	9.50	7.75	11.75
	polymorphs »	13.—	12.25	16.—
	Mono- blasts »	1.50	1.—	2.50
	cytes »			
	Lymfocytes »	7.25	3.50	11.50
	Plasma & Türk cells »	0.25	0.50	0.25
	Smear cells »	3.50	8.50	14.25
	Reticulo endothel. cells »	0.50	3.—	1.—

macro-megalo-cytosis, orthochromia, micro-schizo-poikilocytosis. Erythroblasts 1 in 100 L. Morphology of the leucocytes normal.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 24—7—39 20 cc. liver extract mrk II «Nyco» (8—5—39).

1939	Reticulocytes ‰	Hmglb.	Leuco- cytes	Serum colour	Erythrocytes in millions
22.7	40	48	2900	12	1.20
23.7	37	50	2900		1.26
24.7	26	50	3200		1.61
25.7	15	52	3400		1.57
26.7	29	50	3800		1.52
28.7	50				
29.7	85			6	
30.7	70				
31.7	25	60			1.95
2.8	28	68	4000	4	2.11

*Case 31. H. N. ♂ aged 62. Born 15—7—1874, Elverum. (Ref. nr. 3882/36—37).*

*Occupation:* Captain (Non-commissioned officer).

Since the summer of 1927 he has been breathless on walking up hill and has experienced lassitude and tiredness. Tongue sore since October 1928. His symptoms grew worse, breathlessness in particular being troublesome. He was admitted to the Med. Dept. A. of the Rikshospital 5—2—1929 where pernicious anaemia was diagnosed. Urine: Schlesinger ++. Erythrocytes 1.62 mill. Ewald test meal (after 3/4 hr.) Congo ÷, Uffelmann ÷, ac. 0/4. He recovered under liver treatment and on his discharge was advised to eat 200 g. liver daily. On his readmission 2—8—1933, for relapse, he suffered from his earlier symptoms plus troublesome numbness of his toes. Erythrocytes now 2.3 mill. Since his discharge he had taken 225 g. of pig's liver regularly in the form of rissoles. His symptoms had recurred since August 1936, and he was again admitted to the Med. Dept. A. of the Rikshospital 9—11—1936.

*Present condition:* He looks well and makes no anaemic impression. Tongue moist, clean, very smooth. Resp. unembarrassed. Temp. 37.1. Pulse 84, regular. Blood press. 130/75. Pupils equal and circular, reacting to light and accommodation. Apex beat in fifth intercostal space, 9.5 cm. from the middle line. A systolic blowing murmur over the apex and the second right intercostal space. Slight oedema of the legs which present slight paraesthesia. Patellar reflexes cannot be evoked, the other reflexes normal.

Case 30. Differential cell count of sternal blood.

1939			22—7	27—7
Sternal puncture			asp. easy 0.5 cc. st. blood m.p. +++ f.p. 0 severe a pain	
Erythropoiesis	Erythroblaster	per cent	30.20	52.10
	Leuco-	blasts »		
		cytes »	69.80	47.90
	Promegalomacroblasts	»	2.—	1.—
	Megalobl.	bas. »	15.—	1.—
		eos. »	—	—
	Macrobl.	bas. »	46.—	16.—
		eos. »	29.50	1.50
	Normobl.	bas. »	—	59.50
		eos. »	—	18.—
	Erythrobl.	bas. »	0.50	—
		eos. »	5.50	—
	Erythrobl.	bas. »	1.50	3.—
	division forms	eos. »	—	—
Leucopoiesis	Megacaryocytes	»	—	0.50
	Mast cells immature	»	0.25	0.25
		mature »	—	—
	Eos.	myelocytes »	0.25	0.75
	leucocytes	band forms »	0.25	1.25
		polymorphs »	2.—	1.—
	Myeloblasts	»	2.50	4.25
	Praemyelocytes	»	2.75	4.25
	Neutro-	myelocytes »	10.25	20.25
	philes	young forms »	28.50	23.50
		band forms »	10.75	15.50
		polymorphs »	10.75	11.50
	Mono-	blasts »	} 2.—	0.25
		cytes »		
	Lymphocytes	»	18.50	7.50
	Plasma & Türk cells	»	—	—
	Smear cells	»	1.—	1.50
	Reticulo endothelial cells	»	10.25	7.75

Case 31. Differential leucocyte count of ear blood.

1936		10—11	13—11	16—11	18—11	20—11
Bas. L.	per cent .....	0.5	—	0.5	—	—
Eos. L.	" .....	—	—	2.5	1.5	—
Neutrophils	myelocytes	—	—	0.5	—	—
	young forms	0.5	—	1.0	0.5	—
	band forms	2.0	2.5	2.0	4.5	4.5
	polymorphs	60.5	62.0	57.5	75.0	66.5
Monocytes	" .....	2.0	1.0	1.5	2.5	8.0
Lymphocytes	" .....	34.5	34.5	31.5	16.0	21.0

Case 31. Cell count and Hmglob. from ear and sternal blood.

1936	Reticulocytes per mille		Hmglob. pr. erythrocyt. in $\gamma\gamma$		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
12--11	12									
13--11	9	13	42.—	42.1	72.5	68.—	2.38	2.23	5200	9100
14--11	16									
15--11	18									
16--11	28	64	44.5	38.9	74.4	59.5	2.31	2.11	8000	129000
17--11	76									
18--11	126		38.9		80.—		2.84		7500	
19--11	101									
20--11	41		36.2		83.—		3.13		6900	

The right calf tender on pressure. Urine: Schlesinger (1/10 dilut.) +. Serum colour 13. S. R. 9 mm. after 1 hr. Hb. 73 % = 10,074 g. %. Hb. pr. erythr. 39  $\gamma\gamma$ . Retics. 12 ‰. Erythrocytes 2.38 mill. Leucocytes 5800. Blood smear: eos. 0 %, bas. 0.5 %, m. myelocytes 0.5 %, band forms 2 %, polymorphs 60.5 %, monocytes 2 %, lymphocytes 34.5 %. Marked anisocytosis, macrocytosis, megalocytosis, schizocytosis, microcytosis. Polychromasia, orthochromia.

*Sternal puncture:* Megalo-macro-blastosis. A radiological examination of stomach and duodenum showed normal conditions.

*Treatment:* 13—11—36 10 cc. mrk. E<sup>b</sup> Pb. uprac. Bentz F. = 500 g liver.

Case 31. Differential cell count of sternal blood.

1936			13—11	16—11
Sternal puncture			Asp. easy 0.65 cc. st. blood f.p. +++ m.p. + moderate a. pain	Asp. easy 0.4 cc. st. blood m.p. ++ f.p. +++ severe a. pain
Erythropoiesis	Erythroblasts	per cent	29.—	66.2
	Leuco-	blasts		
		cytes	71.—	33.8
	Promegalomacroblasts	»	6.—	—
	Megalobl.	bas.	—	—
		eos.	—	—
	Macrobl.	bas.	32.—	5.5
		eos.	7.—	1.—
	Normobl.	bas.	40.—	86.5
		eos.	5.—	7.—
	Erythrobl.	bas.	7.—	—
		eos.	3.—	—
	Erythrobl.	bas.	—	—
	division forms	eos.	—	—
Leupopoiesis	Megaearyocytes	»	—	—
	Mast cells	immature	0.5	0.5
		mature	—	—
	Eos.	myelocytes	—	1.25
	leucocytes	band forms	0.5	0.50
		polymorphs	—	0.25
	Myeloblasts	»	1.—	2.—
	Praemyelocytes	»	2.5	5.75
	Neutro-	myelocytes	6.5	8.50
	philes	young forms	9.—	24.—
	philes	band forms	7.5	14.—
		polymorphs	35.—	23.75
	Lymphocytes	»	} 1.5	2.—
	Plasma & Türk cells	»		6.75
	Mono-	blasts	21.—	0.75
		cytes	—	—
	Reticulo endothelial cells	»	—	—
	Smear cells	»	15.—	10.—

Case 32. Cell count and Hmglb. from ear and sternal blood.

1935— 1936	Reticulocytes per mille		Hmglb. pr. erythrocyte, in %		Hmglb.		Erythrocytes in millions		Nucleated blood cells		Sternal blood		Polychromatic erythrocytes		Erythro- blasts in ear blood	Aspirated sternal bl. in cc.
	ear	sternal	ear	sternal	ear	sternal	ear	sternal	ear	sternal	Erythro- blasts per cent	Leuco- cytes per cent	ear	sternal		
15—12	6	43.3	43.3	—	43.—	—	1.37	—	7100	98000	35.—	65.—	1	1	0	—
16—12	2	42.—	43.—	43.—	43.5	39.—	1.42	1.24	8300	98000	35.—	65.—	1	1	0	—
21—12	1	40.5	42.5	42.5	41.5	29.—	1.43	0.94	7900	166000	55.6	44.4	1	2	117	—
22—12	21	51	40.5	40.—	42.5	36.5	1.45	1.27	8400	59200	78.—	22.—	4	2	1330	—
23—12	72	246	39.—	38.5	44.5	36.—	1.56	1.29	12300	208000	75.—	25.—	70	107	3550	—
24—12	210	304	37.—	36.—	46.—	42.5	1.74	1.61	6800	40200	62.2	37.8	118	206	986	—
25—12	—	—	36.—	—	47.—	—	1.81	—	—	—	—	—	—	—	—	—
27—21	103	88	34.—	33.—	61.—	50.—	2.47	2.11	6500	44700	37.3	62.7	28	39	0	—
28—12	46	36.5	—	—	60.—	—	2.26	—	—	—	—	—	—	—	—	—
30—12	13	—	—	—	—	—	2.23	—	—	—	—	—	2	—	0	—
31—12	28	37.—	37.—	—	61.—	—	2.26	—	7600	—	—	—	1	—	0	—
4—1	6	—	—	—	61.—	—	2.25	—	9100	—	—	—	—	—	—	—
6—1	4	36.5	40.5	40.5	63.—	59.5	2.38	2.02	8400	38400	25.5	74.5	1	1	0	—
9—1	11	—	—	—	72.5	—	—	—	8800	—	—	—	1	—	0	—
30—1a	10	39.—	36.—	36.—	75.5	50.5	2.68	1.91	5200	12300	20.—	80.—	1	1	0	0.35
30—1b	11	—	36.—	36.—	44.—	—	—	1.68	—	14020	22.3	77.7	1	1	0	0.35
3—2	6	34.5	28.2	28.2	68.5	45.—	2.73	2.20	6200	153900	32.2	67.8	0	3	0	0.55
4—2	2	36.—	34.—	34.—	69.—	45.—	2.66	1.83	6700	129500	41.—	59.—	1	3	0	0.30
5—2	2	37.5	36.2	36.2	67.—	59.5	2.47	2.27	6400	50300	44.—	56.—	0	5	0	1.80
6—2	10	33.5	32.1	32.1	67.5	70.—	2.67	3.01	7300	32600	36.7	63.3	1	2	0	0.80
7—2	20	34.—	39.1	39.1	68.5	67.—	2.76	2.36	7700	77700	48.6	51.4	3	4	0	0.60
8—2	34	—	37.5	37.5	76.—	78.—	2.87	2.81	7400	144000	48.5	51.5	1	5	0	0.60
10—2	14	36.—	37.3	37.3	74.—	76.—	2.82	2.81	8600	35600	30.8	69.2	0	1	0	0.55
12—2	6	37.—	36.2	36.2	75.—	58.—	2.80	2.23	8000	9150	21.—	79.—	0	0	0	1.10

Case 32. R. S. ♂ aged 59. Born 22—8—1876, Haa, Jaeren. (Ref. nr. 4819/35—36).

Occupation: None (in prison for about 15 years, a couple of years at a time).

His father and several members of his family on his father's side were abnormally fat. He himself has been very fat, his height being 1.64 cm., and his weight at one time over 100 kg. He now weighs 70 kg. Well till 1931 when he began to be troubled by nausea and vomiting in the morning. In the summer of 1935 he began to feel limp, but he continued to do a little work till, a week before admission to the Med. Dept. A. of the Rikshospital, he had to take to his bed. A month ago, folk about him noticed he had become very pale, and since the middle of October he lost 10 kg. Progressive breathlessness since the summer. Two or three months ago he was troubled for some time by soreness of his tongue, a small sore being situated on the tip of it. He has noticed no paraesthesias or paraeses. The actions of bowels and bladder normal.

Present condition: It was noted on 14—12—1935 that he was remarkably pale, with a touch of yellow in his skin. Mucous membranes pale. Pulse

Case 32. Differential leucocyte

1935—1936		20—12	21—12	22—12	23—12	24—12	27—12	30—12	31—12
Pracmyelocytes	per cent	—	0.5	+	1.5	1.0	—	—	—
Bas. L.	»	0.5	0.5	—	0.5	1.0	0.5	1.5	1.0
Eos. L. ....	»	2.5	1.5	3.5	4.0	0.5	4.5	2.0	2.5
Neutrophiles {	myelocytes	0.5	0.5	+	2.5	0.5	—	—	—
	young forms	1.0	2.5	3.0	5.0	8.0	—	—	—
	band forms	4.0	3.5	5.5	7.0	11.0	2.0	1.0	4.0
	polymorphs	63.5	63.0	51.0	41.5	36.5	36.0	55.0	47.0
Monocytes	»	1.0	2.0	1.5	6.0	4.0	10.0	2.5	8.5
Lymphocytes	»	27.0	26.0	35.5	32.0	37.5	47.0	37.5	37.0
Plasma-Türk	»	—	—	—	—	—	—	1.0	0.5
Normobl.	bas. in 200	—	—	10	70	8	—	—	—
	eos. leucocytes	—	—	2	9	25	3	—	—
Megalobl.	bas. »	—	3	6	1	—	—	—	—
	eos. »	—	—	3	—	—	—	—	—
Macrobl.	bas. »	—	—	12	1	1	—	—	—
	eos. »	—	—	5	—	—	—	—	—
Erythrobl.	bas. »	—	—	1	1	—	—	—	—
division forms	eos.	—	—	—	—	—	—	—	—





Case 32. Differential cell

35—36		20—12	21—12	22—12	23—12	24—12	27—12
Erythropoiesis	Erythroblasts per cent	35.—	55.6	78.—	75.—	62.17	37.3
	Leuco- blasts »	65.—	44.4	22.—	25.—	37.83	62.7
	cytes »	—	—	—	—	—	—
	Promegalomacroblasts »	—	—	—	—	—	—
	Megalobl. bas. »	16.4	10.6	0.6	—	—	4.9
	eos. »	3.—	5.4	2.7	—	—	1.3
	Macrobl. bas. »	30.—	66.8	9.4	2.7	1.6	30.1
	eos. »	—	—	2.2	—	—	6.7
	Normobl. bas. »	—	3.6	80.2	69.6	95.9	15.—
	eos. »	—	1.8	4.9	27.7	2.5	42.—
Leucopoiesis	Erythrobl. bas. »	48.6	7.1	—	—	—	—
	eos. »	—	3.9	—	—	—	—
	Erythrobl. bas. »	—	—	—	—	—	—
	eos. »	—	—	—	—	—	—
	division forms eos. »	—	—	—	—	—	—
	Megacaryocytes »	0.25	0.75	—	—	—	0.50
	Mast cells immature »	—	—	—	—	—	—
	mature »	—	0.75	0.25	0.75	0.50	0.50
	Eos. myelocytes »	3.25	1.75	1.75	2.—	0.75	1.—
	leucocytes band forms »	2.—	2.50	0.75	1.75	0.50	2.25
	polymorphs »	1.—	1.25	2.75	3.—	1.—	1.75
	Myeloblasts »	1.50	0.25	0.25	0.75	2.—	1.25
	Praemyelocytes »	8.75	6.50	5.50	7.25	8.75	6.—
	Neutro- myelocytes »	9.—	7.50	5.25	8.50	7.—	9.50
	philes young forms »	9.75	13.75	10.—	16.75	17.—	13.50
	band forms »	28.25	37.50	13.50	30.75	32.—	31.75
	polymorphs »	25.—	16.75	51.25	22.—	22.—	23.—
	Mono- blasts »	} 2.25	1.75	2.25	4.25	1.—	1.75
	cytes »						
	Lymphocytes »	8.—	8.75	6.50	2.25	7.—	6.50
	Plasma & Türk cells »	0.25	—	—	—	0.50	—
	Reticulo endothel. cells »	0.75	0.25	—	—	—	1.—
	Smear cells »	—	—	—	—	—	—

count of sternal blood.

[illegible]

Case 33. O. G. ♂ aged 71. Born 11—12—1866, Heddal, Telemark. (Ref. nr. 5814/37—38).

Occupation: Farmer.

Osteomyelitis at the age of 16. Since then well till about two months ago when he noticed great breathlessness on walking up hill. He suffered more and more from lassitude and giddiness, his appetite flagged, and he experienced a sense of pressure about his heart on exertion. His face has also become much pales, and during the past month he has done no work because he felt he was not up to it. No sores in mouth or on tongue, no abdominal pain. Normal action of bowels and micturition. Admitted for anaemia to the Med. Dept. A. of the Rikshospital 3—1—1938.

*Present condition:* He is pale and yellow and thin. No pain anywhere. Pulse 76, regular. Resp. 16, unembarrassed. Blood press. 135/95. Tongue moist, slightly coated, some atrophic papillae, no sores. A loud systolic blowing murmur over the apex. The second heart sound slightly blurred. Liver and spleen not palpable. Electrocardiogram normal. Ewald test meal (after 3/4 hr.) ac. 0/8, Congo ÷, McLean ÷. Urine: Schlesinger (1/10 dilut.) +. S.R. 18 mm. after 1 hr. Wassermann ÷. Serum colour 5. Retics. 6‰. Hb. 46 % = 6.35 g. %. Hb. pr. erythr. 43.2 γγ. Erythrocytes 1.47 mill. Leucocytes 3400. Blood smear: bas. 1.1 %, eos. 2.9 %, band forms 1.8 %, polymorphs 38.1 %, monocytes 3.5 %, lymphocytes 32.6 %. Considerable anisocytosis, macro-megalo-shizo-poikilo- and microcytosis. Orthochro-

Case 33. Differential leucocyte count of ear blood.

1838	8—1	10—1	11—1	12—1	13—1	17—1	19—1	22—1	14—3
Praemyelocytes per cent	—	0.5	—	0.5	1.5	—	—	—	—
Bas. L. »	—	0.5	—	—	—	1.0	1.0	0.5	—
Eos. L. »	5.0	4.0	2.5	3.0	2.5	6.5	3.0	5.0	13.0
Neutrophiles { myelocytes »	0.5	0.5	4.0	2.0	0.5	1.5	—	—	—
{ young forms »	—	1.0	3.5	—	0.5	—	—	—	—
{ band forms »	5.0	3.0	2.5	2.5	2.0	2.5	2.5	2.0	4.5
{ polymorphs »	53.5	39.5	37.5	40.0	44.0	36.0	51.0	46.5	52.5
Monocytes »	4.0	4.0	5.0	8.0	9.5	13.0	11.5	6.0	5.0
Lymphocytes »	32.0	47.0	45.0	44.0	39.5	39.0	31.0	40.0	24.5
Plasma-Türk »	—	—	—	—	—	0.5	—	—	0.5
Normobl. bas. in 200	—	—	11	12	1	—	—	—	—
eos. leucocytes	—	—	4	19	5	1	—	—	—
Macrobl. bas. »	—	1	1	—	—	—	—	—	—
aaeos. »	—	—	3	1	—	—	—	—	—
Erythrobl. bas. »	—	—	2	—	—	—	—	—	—
eos. »	—	5	6	4	—	—	—	—	—

Case 33. Cell count and Hmglob. from ear and sternal blood.

1938	Reticulocytes per mille		Hmglob.		Erythrocytes in millions		Nucleated blood celles	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
8—1	8	14	46	33 —	1 33	1 11	3100	208100
9—1	6							
10—2	14	16	44	48.—	1.24	1.22	5200	241300
11—1	29	87	45	50.—	1.67	1.43	4600	367000
12—1	131	156	47	48.—	1.50	1.34	5300	296300
13—1	163	281				1.86		191500
14—1	174		52		1.97		4400	
15—1	116	130		66.5		2.05		149500
16—1	88							
17—1	78		56		2.13		4700	
19—1	68		60		2.40		7200	
22—1	20		60		2.20		5200	
27—1	14		69.		2.59		5000	
31—1	8		70		2.78		6100	
3—2	2		73		3.02		4800	
14—3		4	90	87.—	3.72	3.70	7200	37000

masia, no nucleated erythrocytes. Polychromasia less than 1 ‰. Neutrophil over-segmentation.

*Sternal puncture:* Macro-megaloblastosis. Normal conditions found on a radiological examination of stomach and duodenum.

*Treatment:* 8—1—38 2 cc. Pernami forte.

9—1—38 2 cc. „ „

3—2—38 10 cc. Pernami nr. II

4—2—38 10 cc. „ „ II

22—2—38 20 cc. „ „ II.

*Case 34. H. M. ♂ aged 66. Born 9—10—1869, England. (Ref. nr. 5496/35—36).*

*Occupation:* Watchman.

He belongs to a family of four, one member of which died of diabetes mellitus. Well till the summer of 1931 when he suffered from a sense of pressure in the epigastrium. It was worst on an empty stomach, passing off when he took food. His appetite dwindled and he suffered more and more from pallor and lassitude, but not from loss of weight. In the middle of August 1932 his condition became worse, his appetite being wretched. Attacks of vomiting, particularly after exertion. There was a touch of

Case 33. Differential cell count of sternal blood.

1938				8—1	10—1	11—1	12—1	13—1	15—1	14—3
Sternal puncture				Asp. easy 0.7 cc. st. blood m.p. + + + + f.p. 0 severe a. pain	Asp. easy 0.6 cc. st. blood m.p. + + + + + f.p. + severe a. pain	Asp. easy 0.25 cc. st. blood m.p. + + + + + + f.p. 0 severe a. pain	Asp. easy 0 15 cc st.-blood m.p. + + + + + + f.p. 0 severe a. pain	Asp. easy 0.3 cc. st. blood. m.p. + + + + + + f.p. + severe a. pain	Asp. easy 0.3 cc. st. blood m.p. + + + + + + f.p. 0 moderate a. pain	Asp. easy 0.8 cc. st. blood m.p. + + + + + f.p. + + moderate a. pain
Erythropoiesis	Erythroblasts	per cent		50.9	53.5	70.4	67.5	57.1	35.8	13.2
	Leuco- blasts	"		—	—	—	—	—	—	—
	cytes	"		49.1	46.5	29.6	32.5	42.9	64.2	86.8
	Promegalomacrophlasts	"		15.—	2.—	0.25	0.25	0.3	4.5	—
	Megalobl. bas.	"		10.5	—	—	—	—	0.5	—
	eos.	"		—	—	—	—	—	—	—
	Macrobl. bas.	"		65.5	72.5	11.50	9.75	11.—	28.—	8.—
	eos.	"		8.—	1.5	1.50	0.25	0.3	2.5	1.—
	Normobl. bas.	"		—	20.—	81.—	88.—	82.3	45.—	51.—
	eos.	"		—	—	1.—	1.75	5.7	19.5	40.—
	Erythrobl. bas.	"		—	—	—	—	—	—	—
	eos.	"		1.—	2.—	—	—	—	—	—
	Erythrobl. bas.	"		—	2.—	1.75	—	0.4	—	—
	division forms eos.	"		—	—	—	—	—	—	—
Leucopoiesis	Megacaryocytes	"		—	—	+	+	+	—	0.2
	Mast cells	"		—	—	0.25	—	—	—	0.2
	immature	"		—	—	—	—	—	—	—
	mature	"		—	—	—	—	—	—	—
	Eos. myelocytes	"		0.25	2.—	1.25	1.—	0.25	1.50	5.6
	leucocytes	"		2.50	1.50	1.25	1.75	1.50	1.—	2.4
	band forms	"		0.75	1.75	1.—	0.25	0.75	2.—	2.6
	polymorphs	"		3.25	1.75	4.—	1.50	1.50	1.25	1.8
	Myeloblasts	"		3.—	6.50	3.50	5.25	4.—	0.50	2.6
	Praemyelocytes	"		19.50	14.25	19.25	15.25	14.—	13.50	13.—
	Neutro- myelocytes	"		39.—	35.—	31.75	49.—	38.25	32.75	24.2
	philes	"		9.75	7.75	5.50	8.—	6.—	13.75	10.4
	band forms	"		5.50	5.50	10.25	7.25	13.75	13.75	20.4
	polymorphs	"		—	0.25	—	1.—	1.25	1.50	2.6
	Mono- blasts	"		—	—	—	—	—	—	—
	cytes	"		—	—	—	—	—	—	—
	Lymphocytes	"		3.—	3.—	1.50	—	1.50	6.25	5.2
	Plasma & Türk cells	"		—	+	4.—	—	0.50	—	0.6
	Reticulo endothelial cells	"		2.—	—	0.25	0.50	0.50	0.25	0.8
	Smear cells	"		11.50	21.75	16.25	9.25	16.25	12.25	7.4

yellow in his complexion. He suffered more and more from giddiness, headache and dyspnoea when at work. He had therefore to abandon it, and he was admitted to the Med. Dept. A. of the Rikshospital 20—1—1933. He was given campolon and recovered. After discharge he failed to adhere to his liver diet, and he was therefore re-admitted to the Med. Dept. A. 10—1—1936 with his old symptoms. No history of any soreness of the tongue.

*Present condition:* He is pale with a hint of jaundice. Conjunctivae subicteric. Pulse 88, regular, with a suspicion of high tension. Apex beat not palpable. A systolic murmur loudest over the apex and at the left margin of the sternum. Blood press. 160/70. No oedema or rash. Lower border of the liver palpable 1—2 finger-breadths below the costal arch. Temp. on admission 37.5, on discharge, 36.5. Ewald test meal (after 3/4 hr.) ac. 0/2, Congo ÷. Urine: Schlesinger (1/10 dilut.) +. Serum colour 18. S.R. 39 mm. after 1 hr. Hb. 49 % = 6.76 g. %. Hb. pr. erythr. 33 γγ. Erythrocytes 2.05 mill. Leucocytes 4800. Blood smear: eor. 2.5 %, bas. 2.5 %, band forms 2.5 %, polymorphs 62 %, monocytes 4 %, lymphocytes 28.5 %, plasma-Türk 0.5 %. Anisocytosis, poikilocytosis, microcytosis, schizocytosis, macrocytosis, megalocytosis, basophil punctuation, over-segmentation of the neutrophil leucocytes, polychromasia. Retics. 9<sup>0</sup>/<sub>100</sub>.

*Sternal puncture:* Megalo-macroblastosis. A radiological examination showed a normal duodenum and a stomach dislocated over to the right, no tumour infiltration.

*Treatment:* 15—1—36. 15 cc. mrk. BFPH praecip. = 7500 g. liver.

Case 34. Differential leucocyte count of ear blood.

1936		10—1	15—1	16—1	17—1	18—1	19—1	20—1	21—1	22—1	29—1	30—1
Praemyelocytes	per cent	—	—	0.3	0.7	—	0.5	—	—	—	—	—
Bas. L.	"	2.5	—	—	0.3	4.0	1.0	—	—	—	—	—
Eos. L.	"	2.5	0.3	—	1.0	—	1.0	2.0	3.0	1.5	1.0	3.0
Neutrophils	myelocytes	—	0.7	—	0.7	1.0	—	—	—	—	—	—
	young forms	—	0.7	0.7	1.7	2.0	1.0	1.5	1.5	—	—	—
	band forms	2.5	5.0	2.3	2.3	2.0	2.5	2.0	1.0	1.0	4.0	3.5
	Polymorphs	59.5	53.3	49.4	55.0	55.5	49.5	52.5	49.0	51.0	56.0	58.5
Monocytes	"	4.0	3.7	5.0	4.0	3.5	8.5	6.0	5.5	6.0	6.5	4.0
Lymphocytes	"	28.5	36.3	42.3	34.3	32.0	36.0	36.0	40.0	40.5	32.5	30.0
Plasma-Türk	"	0.5	—	—	—	—	—	—	—	—	—	—
Megalobl.	bas. in 200 leuco-	4	1	—	—	—	—	—	—	—	—	—
	eos. cytes	—	1	2	1	3	—	1	—	—	—	—
Normobl.	bas.	—	1	1	4	—	1	—	—	—	—	—
	eos.	—	—	1	8	25	12	3	1	—	—	—
Macrobl.	bas.	—	—	—	1	—	—	—	—	—	—	—
	eos.	—	—	1	—	—	—	—	—	—	—	—

Case 34. Cell count and Hmgbl. from ear and sternal blood.

1936	Reticulocytes per mille		Hmgbl. per erythrocyte in $\gamma\gamma$		Hmgbl.		Erythrocytes in millions		Nucleated blood cells		Sternal blood		Erythroblasts in earblood
	ear-blood	sternal-blood	ear-blood	sternal-blood	ear-blood	sternal-blood	ear-blood	sternal-blood	ear-blood	sternal-blood	erythroblasts per cent	leucocytes per cent	
10-1	9		33.—		49.—		2.05		4800				96.—
15-1	6	11	32.2	35.6	45.5	39.5	1.95	1.53	5500	77600	57.8	42.4	54.—
16-1	11	14	35.6	36.7	48.—	34.—	1.86	1.28	4300	37500	62.7	37.3	70.—
17-1	17	23	33.6	38.—	47.5	35.—	1.95	1.27	7400	165400	78.7	21.3	350.—
18-1	51	74	33.4	35.7	45.5	40.5	1.85	1.56	8100	177400	79.3	20.7	1260.—
19-1	198		37.—		48.—		1.79		5500				357.5
20-1	407	486	30.9	38.4	57.—	31.—	2.51	1.14	4200	10800	53.2	36.8	88.—
21-1	229	233	31.3	32.5	60.5	45.5	2.67	1.93	5000	57000	61.9	38.1	25.—
22-1	162	200	31.5	32.—	60.—	48.—	2.63	2.07		37400	59.3	40.7	0.—
23-1	97												
24-1	83												
29-1	19		28.4		66.—		3.21		6000				
4-2	9		27.7		75.—		3.71		6000				

Case 34. Differential cell count of sternal blood.

1936			15—1	16—1	17—1	18—1	20—1	21—1	22—1
Erythropoiesis	Erythroblasts	per cent	57.8	62.9	78.7	79.3	63.2	61.9	59.3
	Leuco-								
	blasts	»							
	cytes	»	42.2	37.1	21.3	20.7	36.8	38.1	40.7
	Promegalomacroblasts	»	7.—	—	—	—	—	—	—
	Malobl.	bas.	10.3	3.—	—	—	—	0.2	0.4
		cos.	3.7	0.5	—	0.4	—	—	—
	Macrobl.	bas.	16.7	15.8	4.—	3.3	0.8	1.4	1.7
		cos.	2.—	0.7	0.2	0.4	—	—	0.7
	Normobl.	bas.	—	4.6	91.2	63.3	34.7	88.4	71.7
		cos.	—	1.—	1.5	32.6	64.5	10.—	25.8
	Erythrobl.	bas.	58.7	72.1	—	—	—	—	—
		cos.	—	—	—	—	—	—	—
	Erythrobl.	bas.	1.6	2.3	3.—	—	—	—	—
Leucopoiesis	division forms	cos.	—	—	—	—	—	—	—
	Megacaryocytes	»	0.25	—	—	—	—	—	—
	Mast cells	immature	—	0.25	—	—	0.3	—	—
		mature	0.25	—	—	—	—	—	0.50
	Eos.	myelocytes	1.75	1.25	2.—	1.75	0.3	0.75	0.75
	leucoocytes	band forms	2.—	3.25	1.—	0.50	0.7	1.25	—
	cytes	polymorphs	2.25	1.25	1.—	2.25	2.3	1.25	0.50
	Myeloblasts	»	1.—	—	—	0.25	—	0.50	0.25
	Praemyelocytes	»	11.25	4.50	7.—	12.75	7.3	6.75	6.—
	Neutro-	myelocytes	7.75	8.75	12.3	9.25	6.—	8.75	10.—
	philes	young forms	28.75	20.—	26.3	18.75	20.3	21.50	32.25
		band forms	19.50	19.50	34.2	35.—	22.4	31.50	22.50
		polymorphs	17.50	37.25	15.3	18.25	34.4	24.50	22.50
	Mono-	blasts	1.25	0.75	0.3	0.25	—	0.75	1.75
		cytes	—	—	—	—	—	—	—
	Lymphocytes	»	5.—	2.25	0.3	0.50	6.—	2.—	3.—
	Plasma & Türk cells	»	1.—	1.—	0.8	0.50	—	0.50	—
	Reticulo endothelial cells	»	0.50	—	—	—	—	—	—
	Smear cells	»	—	—	—	—	—	—	—

Case 35. K. G. K. ♂ aged 72. Born 10—11—1863, Oslo. (Ref. nr. 7588/35—36).

Occupation: Pensioner.

In 1898 contracted syphilis, and in 1912 developed syphilitic sores in his mouth, being given effective treatment with mercury on both occasions. In the autumn of 1935 sores again in his mouth, was treated at the Fourth Dept. of Ullevaal Hospital with neosalvarsan and wismol. Since October



Case 35. Differential leucocyte count of ear blood.

1936		30-3	2-4	3-1
Bas. L.	.....		2.0	0.5
Eos. L.	.....	3.5	2.5	2.5
Stemophiles	myelocytes	-	0.5	-
	band forms	5.0	2.5	2.0
	polymorphs	45.5	47.0	46.5
Monocytes	.....	1.0	1.0	1.0
Lymphocytes	.....	45.0	44.5	47.5
Erythrobl. bas. in 200 leucocytes	.....	-	2	-

Case 35. Cell count and Hmglb. from ear and sternal blood.

1936	Reticulocytes per mille		Hmglb.		Erythrocytes in millions		Nucleated blood cells		Erythroblast in earblood per cent
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	
28-3	5								
29-3	9								
30-3	5	8	60	51.5	2.27	2.74	5800	42400	0
31-3	2								
1-4	2								
2-4	18	18	62.5	52.	2.16	1.83	1900	34000	490
3-4	62	106	67.	59.	2.27	2.10	5100	13800	0
4-4	78								
5-4	150								
6-4	118		67.		2.19		8500		
8-4	42		70.		2.72		7400		
15-4	44		76.		3.16		9000		
20-4	5		81.		3.82		7600		

1934, increasing pallor and lassitude, but without any definite sense of illness. A strifle giddy at Christmas time, appetite poor, some loss of weight. Admitted to the Eye Dept. of the Rikshospital 17-3-1935 for cataract. Transferred 26-3-1935 to the Med. Dept. A with the diagnosis of pernicious anaemia.

*Present condition:* He is thin and very pale, with a hint of yellow in his skin. Mucous membranes also pale. Tongue moist, smooth, sore, its lining atrophic. Temp. 37.2. Pulse 72, regular. Blood press. 150/90. Pupils react to light and accommodation. No demonstrable apex beat, no cardiac dullness, heart sounds clear. Plantar reflexes inverted, Achilles reflexes absent, gait a trifle unsteady. Liver dullness from 6th rib to a point 3 finger-breadths below the costal arch, the margin of the liver being firm and

Case 35. Differential cell count of sternal blood.

1936		30—3	2—4	3—4
Sternal puncture		Asp. easy 0.65 cc. st. blood m.p. +++ f.p. +	Asp. easy 0.85 cc. st. blood m.p. +++ f.p. +	Asp. easy 0.2 cc. st. blood m.p. +++ f.p. +
Erythropoiesis	Erythroblasts	23.6	55.6	47.3
	Leuco- blasts			
	cytes	76.4	44.4	52.7
	Promegalomacroblasts	10.—	—	—
	Megalobl. bas.	16.3	1.4	0.3
	cos.	3.4	0.4	—
	Macrobl. bas.	28.—	4.7	3.2
	cos.	2.5	—	0.3
	Normobl. bas.	7.6	75.9	70.5
	cos.	—	15.8	24.—
	Erythrobl. bas.	17.—	—	—
	cos.	12.7	—	0.3
	Erythrobl. bas.	2.5	1.4	0.7
	division forms cos.	—	0.4	0.7
Leucopoiesis	Megacaryocytes	0.25	0.50	—
	Mast cells immature	0.25	0.50	0.25
	mature	0.25	0.75	0.25
	Eos. myelocytes	0.25	1.—	2.—
	leucocytes band forms	3.50	1.—	3.—
	polymorphs	1.75	3.50	—
	Myeloblasts	0.50	1.50	2.25
	Praemyelocytes	6.25	7.75	3.50
	Neutro- myelocytes	8.—	7.50	10.75
	philes young forms	19.—	10.75	28.—
	band forms	15.25	13.50	19.—
	polymorphs	27.75	36.25	12.50
	Mono- blasts	0.25	3.25	2.50
	cytes			
	Lymphocytes	11.—	9.75	8.—
	Plasma & Türk cells	0.50	—	0.25
	Reticulo endothelial cells	1.25	—	—
	Smear cells	4.—	2.50	7.50

smooth on palpation. Urine: Schlesinger (1/10 dilut.) ++. Serum colour 7. S.R. 28 mm. after 1 hr. Ewald test meal (after 3/4 hr.) ac. 0/4, Congo ÷, McLean ÷. Wassermann ++. Hb. 60 % = 8.28 g. %. Hb. pr. erythr. 36.5 γγ. Erythrocytes 2.27 mill. Retics. 5 %<sub>100</sub>. Leucocytes 6600. Blood

smear: eos. 3.5 %, band forms 5 %, polymorphs 45.5 %, monocytes 1 %, lymphocytes 45 %. Just a hint of over-segmentation of the nuclei of the neutrophil cells. Marked anisocytosis, Faint anisochromia. Microcytosis, schizocytosis, poikilocytosis, macro-megalocytosis.

*Sternal puncture:* Megalo-macroblastosis. A radiological examination showed sclerosis of the aorta and emphysema of the lungs.

*Treatment:* 30—3—36. 24 cc. B. F. salt abstracted according to D. and W. = 2400 g. liver.

*Case 36. N. R. M. ♂ aged 74. Born 14—9—1863, Tinn. (Ref. nr 2412/37—38).*

On the whole well till 1932 when he was confined to bed for a month by pneumonia. His convalescence was slow on account of lassitude, tinnitus, giddiness and throbbing in his head. His skin was pale yellow, and his legs ached considerably. Cannot remember any soreness of his tongue. After being up and about but feeling poorly for a couple of months, he suffered so much at last from lassitude that he had to keep to his bed most of the day. A doctor prescribed a diet of liver. Striking improvement after only eight days, and in a month he was fit for work. Since then he has occasionally suffered from lassitude and tiredness, but has, on the whole, kept fit till about a fortnight ago when he suddenly developed nausea and diarrhoea. Since then he has suffered more from lassitude and has had his previous symptoms, but no soreness of his tongue. Appetite wretched, and apart from the attack of diarrhoea he has been inclined to be constipated. Troublesome frequency of micturition. Loss of weight (about 11 kg.) Admitted to the Med. Dept. A. of the Rikshospital 15—9—1937.

Case 36. Cell count and Hmglb. from ear and sternal blood.

1937	Reticuloeytes per mille	Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	earblood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
18—9	21	24	26	0.95	0.92	3700	64200
20—9	78	23		0.88		8000	
21—9	134						
22—6	315	27	30	1.10	1.25	12500	175100
23—9	580						
24—9	256	37		1.95		3500	
25—9	230						
26—9	210						
27—6	70	49		2.17		1000	
29—9	10	52		2.29		3000	

Case 36. Differential cell count of sternal blood.

1937			18—9	22—9
Sternal puncture			Asp. easy 0.8 cc. st. blood m.p. ++ f.p. ÷ a. pain	Asp. easy 0.75 cc. st. blood m.p. ++ f.p. 0 severe a. pain
Erythropoiesis	Erythroblasts	per cent	48.5	71.7
	Leuco-      blasts	»		
	cytes	»	51.5	28.3
	Promegalomacroblasts	»	22.5	0.5
	Megalobl.    bas.	»	2.—	—
	eos.	»	—	—
	Macrobl.    bas.	»	60.—	12.5
	eos.	»	8.—	—
	Normobl.    bas.	»	—	85.—
	eos.	»	—	1.—
	Erythrobl.    bas.	»	4.5	—
	eos.	»	—	—
	Erythrobl.    bas.	»	3.—	1.—
	division forms eos.	»	—	—
Leucopoiesis	Megacaryocytes	»	—	—
	Mast cells    immature	»	0.25	0.2
	mature	»	0.25	0.4
	Eos.          myelocytes	»	3.50	1.2
	leucocytes    band forms	»	1.50	1.—
	polymorphs	»	2.50	4.—
	Myeloblasts	»	2.50	2.4
	Praemyelocytes	»	3.75	2.8
	Neutro-      myelocytes	»	18.—	17.—
	philes        young forms	»	37.—	29.—
	band forms	»	2.50	8.2
	polymorphs	»	6.—	11.6
	Mono-        blasts	»	0.75	} 0.2
	cytes	»	0.50	
	Lymphocytes	»	2.25	4.—
	Plasma & Türk cells	»	—	0.2
	Reticulo endothelial cells	»	1.75	0.2
	Smear cells	»	17.50	17.—

*Present condition:* He is thin and pale yellow. Pulse 100, regular. Temp. 38.2. Blood press. 120/90. Tongue dry, not coated, and neither smooth nor sore. Apex beat 9.5 cm. from the middle line in the 5th intercostal space. Cardiac diam. 13 cm. A faint and rather coarse systolic murmur loudest

over the second right intercostal space. Accentuation of the second heart sound. Liver dulness from 6th rib to a point 2 finger-breadths below the costal arch where its border is palpable. Spleen not palpable. Reflexes normal. Ewald test meal (after 3/4 hr.) Congo  $\div$ , McLean  $\div$ , ac. 0/8. Urine: Schlesinger (1/10 dilut.)  $++$ . Wassermann  $\div$ . S.R. 85 mm. (after 1 hr.). Serum colour 8. Retics. 22 %<sub>100</sub>. Hb. 24 % = 3.31 g. %. Hb. pr. erythr. 34.8  $\gamma\gamma$ . Erythrocytes 0.95 mill. Leucocytes 3700. Blood smear: eos. 0.9 %, band forms 12.6 %, polymorphs 40.3 %, monocytes 3.4 %, lymphocytes 42.8 %. Neutrophil over-segmentation. Macro-megalo-cytosis, anisocytosis, microcytosis, schizocytosis, poikilocytosis, polychromasia, basophil punctuation. Erythroblasts 8/200 L.

*Sternal puncture:* Megalo-macroblastosis. Nothing abnormal found on a radiological examination of stomach and duodenum.

*Treatment:* 18—9—37 Liver injection.

*Case 37, L. H. ♂ aged 35. Born 12—8—1900, Hurum. (Ref. nr. 6308/35—36).*

*Occupation:* Electrician.

Pleurisy in 1920, progressive, chronic polyarthritis in 1916. Troubled since 1922 by dragging and burning pain in the epigastrium. At first it came in bouts, but of recent years it has occurred daily. Admitted to the Med. Dept. A. of the Rikshospital for polyarthritis 5—2—1936.

Case 37. Cell count and Hmglob. from ear and sternal blood.

1936	Reticulocytes per mille		Hmglob. pr. erythroc. in $\gamma\gamma$		Hmglob.		Erythrocytes in millions		Nucleated blood cells		Aspirated sternal blood in cc
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	
24—2	6	31	38.4	51.6	75	72.5	2.69	1.91	5600	104800	0.6
25—2	31										
26—2	36	48	41.—	40.4	79	77.—	2.66	2.63	4400	63000	0.7
27—2	64										
28—2	60		38.1		72		2.61		5700		
29—2	110										
1—3	99		38.—		78		2.83		6600		
2—3	81										
3—3	70		37.6		87		3.19		4800		
4—3	65										
5—3	51		37.6		95		3.49		7000		
11—3	8		31.6		97		4.24		7400		
14—3	12		32.5		100		4.26		5900		
26—3			30.1		112		5.14				
16—4			31.9		130		5.63				

Case 37. Differential cell count of sternal blood.

1936			24—2	26—2
Erythropoiesis	Erythroblasts	per cent	32.—	42.7
	Leuco- blasts	»		
	cytes	»	68.—	47.3
	Promegalomacroblasts	»	0.5	—
	Megalobl. bas.	»	5.7	2.3
	eos.	»	5.7	2.7
	Macrobl. bas.	»	46.3	14.1
	eos.	»	—	—
	Normobl. bas.	»	6.8	35.9
	eos.	»	9.4	35.9
	Erythrobl. bas.	»	7.3	4.7
	cos.	»	12.—	1.9
Leucopoiesis	Erythrobl. bas.	»	6.3	1.2
	division forms eos.	»	—	1.2
	Megacaryocytes	»	—	—
	Mast cells immature	»	0.50	—
	mature	»	—	—
	Eos. myelocytes	»	3.—	2.25
	leucocytes band forms	»	2.25	0.75
	polymorphs	»	1.75	2.25
	Myeloblasts	»	2.25	1.75
	Praemyelocytes	»	2.75	5.—
	Neutro- myelocytes	»	7.25	11.25
	philes young forms	»	24.75	19.50
	band forms	»	26.50	26.75
	polymorphs	»	21.—	23.50
	Mono- blasts	»	0.75	0.75
	cytes	»		
	Lymphocytes	»	6.—	6.—
	Plasma & Türk cells	»	—	—
	Reticulo endothelial cells	»	1.25	0.25
	Smear cells	»	—	—

*Present condition:* State of general nutrition medium, not particularly pale. Temp. 37.6, pulse 76, regular. Blood press. 110/70. Pupils normal. Tongue moist, clean, sores on both sides of the middle line. Arthritic changes in most of his joints. Electrocardiogram normal. Urine: (1/10 dilut.) Schlesinger ÷. Ewald test meal (after 3/4 hr.) ac. 0/4. Congo ÷, McLean ÷. S.R. 135 mm. after 1 hr. Serum colour 3. Wassermann ÷. Hb. 68 % = 9.38 g. %. Erythrocytes 2.63 mill. Leucocytes 6600. Blood smear: metamyelocytes 1 %, band forms. 27 %, polymorphs 44 %, lymphocytes 25 %,

monocytes 3 %. Polychromasia, marked anisocytosis, microcytosis and schizocytosis, Anisochromia, macrocytosis. Toxic neutrophil granulation and nuclear structure.

24—2—1936. Serum colour 3. Urine: Schlesinger (1/10 dilut.) +. Hb. 75 % = 10.35 g. %. Hb. pr. erythr. 38 γγ. Erythrocytes 2.69 mill. Leucocytes 5600. Retics. 6‰. Blood smear: bas. 0.5 %, band forms 10.5 %, polymorphs 48.5 %, monocytes 3 %, lymphocytes 37.5 %. Polychromasia, marked anisocytosis, microcytosis and schizocytosis, anisochromia, macrocytosis. Toxic neutrophil granulation and nuclear structure.

*Sternal puncture:* Megalo-macroblastosis. Normal conditions found on radiological examination of stomach and duodenum.

*Treatment:* 24—2—36 }  
25—2—36 } a total of 100 cc. Pernami  
26—2—36 }

*Case 38. H. F. ♀ aged 59. Born 27—12—1888, Lilleström. (Ref. nr. 6794/37—38).*

*Occupation:* Widow.

Since about 1931 she has noticed she has become remarkably pale and tired. Skin slightly yellow and urine occasionally very dark. Heart excitable on exertion. Loss of appetite. After heavy food a slight sensation of soreness in her tongue. Admitted to the Med. Dept. A. 15—12—1935 when pernicious anaemia was diagnosed. Hb. 28 %. Erythrocytes 1.38 mill. Leucocytes 3200. She was treated with liver and discharged as perfectly well 12—3—1934.

Case 38. Cell count and Hmglb. from ear and sternal blood.

1938	Reticulocytes per mille		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
1—2	22	24	27	26	0.91	0.77	4200	101500
2—2	24							
3—2	20		26		0.93		5000	
4—2	46		26		0.96		4400	
5—2	162	450	29	31	1.04	1.39	4700	157400
6—2	418							
7—2	241							
8—2	142		45		1.87		3200	
12—2	52		52		2.40		4400	
16—2	32		60		2.45		4400	
25—2			67		2.96		5500	
28—2			66		2.66			

Case 38. Differential cell count of sternal blood.

1918			1—2	5—2
Sternal puncture			Asp. under vac. [10 cc.] 0.6 cc. st. blood m.p. +++++ f.p. 0 severe a. pain	Asp. easy 0.5 cc. st. blood m.p. +++++ f.p. 0 severe a. pain
Erythropoiesis	Erythroblasts	per cent	44.1	69.4
	Leuco- blasts	»		
	cytes	»	55.9	30.6
	Promegalomacroblasts	»	18.5	1.2
	Megalobl. bas.	»	5.—	—
	eos.	»	—	—
	Macrobl. bas.	»	70.5	11.8
	eos.	»	3.5	1.—
	Normobl. bas.	»	—	69.2
	eos.	»	—	16.2
	Erythrobl. bas.	»	0.5	—
	eos.	»	0.5	—
	Erythrobl. bas.	»	1.5	0.6
	division forms eos.	»	—	—
Leucopoiesis	Megacaryocytes	»	+	+
	Mast cells immature	»	0.50	0.25
	mature	»	—	—
	Eos. myelocytes	»	5.25	3.—
	leucocytes band forms	»	5.—	2.75
	polymorphs	»	3.75	1.50
	Myeloblasts	»	0.75	5.25
	Praemyelocytes	»	2.50	8.75
	Neutro- myelocytes	»	16.75	19.—
	philes young forms	»	23.25	25.—
	band forms	»	5.—	7.25
	polymorphs	»	8.75	5.—
	Mono- blasts	»	2.—	0.50
	cytes	»		
	Lymphocytes	»	5.75	0.50
	Plasma & Türk cells	»	—	—
	Reticulo endothelial cells	»	2.—	1.—
	Smear cells	»	18.75	20.25



Latterly liver treatment has been inadequate, so her lassitude has increased, and she is disinclined for walking. Tongue sore, and of late her fingers have seemed to be numb. Admitted to the Med. Dept. A. of the Rikshospital 1—2—1938.

*Present condition:* She is pale and her skin is definitely yellow. Mucous membranes almost colourless, Pulse 180, regular. Resp. 24, unembarrassed. Tongue moist, clean, smooth. Temp. 37.3. Blood press. 125/50. Total cardiac diam. 16.5 cm. Systolic blowing murmur over the whole of her heart. Liver palpable 3 finger-breadths below the costal arch. Spleen palpable. Urine: (1/10 dilut.) Schlesinger ++. Serum colour 9. S.R. 50 mm. after 1 hr. Wassermann ÷. Retics. 22 %<sub>100</sub>. Hb. 27 % = 3.73 g. %. Hb. pr. erythr. 40.9  $\gamma\gamma$ . Erythrocytes 0.91 mill. Leucocytes 4200. Blood smear: eos. 6.5 %, myelocytes 0.6 %, metamyelocytes 1.3 %, band forms 5.8 %, polymorphs 40 %, monocytes 3.9 %, lymphocytes 41.9 %. Marked anisocytosis, macrocytosis, megalocytosis, schizocytosis, poikilocytosis, orthochromasia, microcytosis, polychromasia about 1 %<sub>100</sub> Over-segmentation of the nuclei of the neutrophil leucocytes.

*Sternal puncture:* Macro-megalocytosis.

*Treatment:* 1—2—38 20 cc. Pernami nr. 08—09—10.

2—2—38 20 cc. Pernami nr. 08—09—10.

28—2—38 20 cc. Pernami nr. 08—09—10.

*Case 39. E. K. ♀ aged 33. Born 10/2—1894, Fana. (Ref. nr. 82/37—38).*

*Occupation:* Wife of agent.

Well till 1920 when she was operated on for a tumour of the ovary. After suffering for many years from lassitude and frequent attacks of fainting, she was admitted to the Seventh Dept. of the Ullevaal Hospital where pernicious anaemia was diagnosed. Hb. 39 %. Erythrocytes 1.5 mill. Under liver treatment she attained to hb. 80 % in about 7 weeks, the erythrocytes reaching 3.93 mill. Since her discharge she has almost daily eaten 200 g. liver, half of which was raw. In 1929 she was in the Vestfold County hospital, treated for «liver poisoning» (always watery stools). In 1931 she was treated in the Med. Dept. A. of the Rikshospital for neurasthenia. Ever since her discharge from hospital she has been given fortnightly injections of Pernami. Since April 1937 she has suffered more from lassitude, and a fortnight before re-admission to hospital she became breathless when out walking, feeling giddy and noticing irregular heart beats. She consulted a doctor, and a similar attack overtaking her some days later, she was admitted to hospital for observation of her heart. Micturition, menstruation and the action of her bowels had been normal, but she had been somewhat troubled by slight smarting of the tip of her tongue. Admitted to the Med. Dept. A. of the Rikshospital 3—7—1937.

*Present condition:* No sign of heart disease to be found. S.R. 6 mm.

Case 39. Differential cell count of sternal blood.

1937				6—9
Erythropoiesis	Erythroblasts	per cent	.....	12.4
	Leuco-      blasts	»	.....	87.6
	cytes	»	.....	—
	Promegalomacroblasts	»	.....	2. —
	Megalobl.      bas.	»	.....	—
	eos.	»	.....	—
	Makrobl.      bas.	»	.....	7. —
	eos.	»	.....	—
	Normobl.      bas.	»	.....	48. —
	eos.	»	.....	43. —
	Erythrobl.      bas.	»	.....	—
	eos.	»	.....	—
	Erythrobl.      bas.	»	.....	—
	division forms eos.	»	.....	—
	Megacaryocytes	»	.....	+
Leucopoiesis	Mast cells      immature	»	.....	0.25
	mature	»	.....	—
	Eos.            myelocytes	»	.....	0.75
	leucoocytes    band forms	»	.....	1. —
	polymorphs	»	.....	1. —
	Myeloblasts	»	.....	0.25
	Praemyelocytes	»	.....	1.25
	Neutro-      myelocytes	»	.....	13.50
	philes        young forms	»	.....	37.50
	band forms	»	.....	12. —
	polymorphs	»	.....	17.50
	Mono-        blasts	»	.....	}
	cytes	»	.....	
	Lymphocytes	»	.....	7.75
	Plasma & Türk cells	»	.....	0.25
	Smear cells	»	.....	7. —
	Reticulo endothelial cells	»	.....	—

Wassermann ÷. Serum colour 3.5. Urine: Schlesinger (1/1) +. Hb. 95 %—13.11 g. %. Hb. pr. erythr. 29.1  $\gamma$ . Erythrocytes 4.5 mill. Leucocytes 4300. Blood smear: eos. 2.5 %, metamyelocytes 0.5 %, band forms 2.5 %, polymorphs 43.5 %, monocytes 6 %, lymphocytes 45 %. Slight anisocytosis, orthochromia, many small haemoglobinrich erythrocytes. No macrocytes. Marked pathological granulation of the nuclear structure of the neutrophil leucocytes. No over-segmentation of the leucocytes.

*Sternal puncture:* Macro-normoblastosis.

Case 40. O. T. ♀ aged 39. Born 31—12—1897, Brandbu. (Ref. nr 3647/37—38).

Occupation: Wife of a mechanic.

Well till the spring of 1936 when she had a period of lassitude, was quite pale, and suffered from soreness of her tongue. Fleeting pains in arms and legs, but no paraesthesias. Recovered completely after 16 injections given by a doctor in the course of 16 weeks. Recovery maintained till the beginning of October 1937 when she began to suffer from lassitude, pricking and stabbing in her legs and, latterly, also in her arms. Though suffering from tiredness and giddiness, she was not confined to bed. During the last few days soreness of her tongue. Her appetite has been good, and she has been able to tolerate all kinds of food, and has not lost weight. Slightly constipated, micturition normal. The doctor sending her to hospital stated that she had received 3 cc. of Pernami per week, the last injection having been given a fortnight earlier. She was admitted to the Med. Dept. A. of the Rikshospital 22—10—1937.

*Present condition:* She is rather fat but not pale. Temp. 37.8. Pulse 76, regular. Resp. 16, unembarrassed. Blood press. 120/90. Electrocardiogram normal. Liver and spleen not enlarged. S.R. 29 mm. Ewald test meal (after 3/4 hr.) Congo ÷, McLean ÷, ac. 0/7. Serum colour 4. Urine: Schlesinger (1/10 dilut.) +. Hb. 84 % = 11.59 g. %. Hb. pr. erythr. 37.8 γγ. Erythrocytes 3.07 mill. Leucocytes 6700. Retics. 8 %<sub>100</sub>. Wassermann ÷. Blood smear: bas. 0.9 %, eos. 7.1 %, band forms 6.3 %, polymorphs 33 %, monocytes 2.4 %, lymphocytes 15.3 %. Macro-(megalo?) cytosis. A few schizocytes, microcytes and poikilocytes. Polychromasia. A single neutrophil leucocyte shows over-segmentation of its nucleus.

*Sternal puncture:* Macro-normoblastosis. Normal findings on a radiological examination of stomach and duodenum.

*Treatment:* 25/28—10—37 40 cc. Pernami nr. 3.

5—11—37 50 cc. Pernami nr. 3.

22/24—11—37 30 cc. campolon.

1/20—12—37 200 g. liver per day.

Case 40. Cell count and Hmg/b. from ear and sternal blood.

1937	Reticulocytes per mille		Hmg/b.		Erythrocytes in millions		Nucleated blood-cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
23—10	8		84		3.07		6700	
6—11	10		85		3.10		5800	
15—11	12		87		3.43		7600	
22—11	12		87		3.57		7300	
24—11	12	42		85		3.31		131700
28—12			94		4.37		9300	

Case 40. Differential cell count of sternal blood.

1937			24—11
Sternal puncture			Asp. easy 0.7 cc. st. blood m.p. + + + + f.p. + severe a. pain
Erythropoiesis	Erythroblasts	per cent .....	36.7
	Leuco-      blasts	" .....	
	cytes	" .....	63.3
	Promegalomacroblasts	" .....	—
	Megalobl.      bas.	" .....	—
	eos.	" .....	—
	Macrobl.      bas.	" .....	17.—
	eos.	" .....	—
	Normobl.      bas.	" .....	78.—
	eos.	" .....	4.—
	Erythrobl.      bas.	" .....	—
	eos.	" .....	—
	Erythrobl.      bas.	" .....	1.—
	division forms eos.	" .....	—
Leucopoiesis	Megacaryocytes	" .....	+
	Mast cells      immature	" .....	0.5
	mature	" .....	—
	Eos.            myelocytes	" .....	1.75
	leucoocytes      band forms	" .....	0.75
	polymorphs	" .....	0.25
	Myeloblasts	" .....	1.—
	Praemyelocytes	" .....	4.—
	Neutro-      myelocytes	" .....	19.25
	philes      young forms	" .....	36.75
	philes      band frms	" .....	9.50
	polymorphs	" .....	12.25
	Mono-      blasts	" .....	} 0.25
	cytes	" .....	
	Lymphocytes	" .....	7.25
	Plasma & Türk cells .....	" .....	0.75
	Reticulo endothelial cells	" .....	—
	Smear cells	" .....	5.75

*Case 41. K. K. ♀ aged 48. Born 3—8—1889, Trysil. (Ref. nr. 7074/37—38).*

*Occupation: Nurse.*

In December 1932 she suffered from fleeting pains in her legs, progressive lassitude, thirst and breathlessness on exertion. A doctor diagnosed anaemia, and she began to eat liver. Her strength returned to some extent, but there was no change in the pain in her legs, and she was as thirsty as ever. A year later diabetes was diagnosed, and she improved greatly under treatment for it. Latterly she has suffered from some soreness of her tongue and from periodic paraesthesias of fingers and toes. Since Christmas 1932 has eaten liver daily (300 g.) for 1 ½ year. She ceased to do so in the summer of 1933, but off and on has eaten some liver up to the present time, an average of 150—200 g. per week. She was admitted to the Med. Dept. A. of the Rikshospital for the control of her diabetes and pernicious anaemia 8—2—1938.

*Present condition:* In moderately good general condition. Complexion fresh. Sclerae subicteric. Pulse 82, regular. Temp. 38. Tongue moist, clean, a trifle smooth. Resp. unembarrassed. Blood press. 130/80. Cardiac diam. 13.5 cm. A systolic murmur over the heart, loudest over the sternum. Liver not definitely palpable. Ewald test meal (after 3/4 hr.) ac. 0/4. Congo ÷, McLean ÷, Wassermann ÷. Serum colour 5. S.R. 115 mm. Retics. 4 ‰. Hb. 76 % = 10.49 g. %. Hb. pr. erythr. 31.9 γγ. Erythrocytes 3.29 mill. Leucocytes 7600. Blood smear: bas. 0.7 %, eos. 4.2 %, band forms 7 %, polymorphs 45.4 %, monocytes 9.8 %, lymphocytes 32.9 %. Considerable anisocytosis and macro-megalo-cytosis. Microcytosis, schizocytosis, orthochromasia, poikilocytosis, polychromasia, no over-segmentation of the nuclei of the neutrophil leucocytes.

*Sternal puncture:* Macro-normoblastosis.

*Treatment:* 24—2—38 10 cc. Pernami nr. II.

25—2—38 10 cc. Pernami nr. II.

12—3—38 20 cc. Pernami nr. 12.

Case 41. Cell count and Hmglob. from ear and sternal blood.

1938	Reticulocytes per mille		Hmglob.		Erythrocytes in million		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
9—2			76		3.29		7600	
23—2	30		72		3.39		5900	
24—2	34	90		69		2.57		86100
27—2	72							
25—3	4		87		4.30		10000	

Case 41. Differential cell count of sternal blood.

1938			24—2
Sternal puncture			Asp. easy 0.9 cc. st. blood m.p. ++ f.p. traces Moderate a. pain
Erythropoiesis	Erythroblasts	per cent .....	26.3
	Leuco- blasts	» .....	
	cytes	» .....	73.7
	Promegalomacroblasts	» .....	1.—
	Mcgalobl. bas.	» .....	—
	cos.	» .....	—
	Macrobl. bas.	» .....	44.—
	eos.	» .....	13.—
	Normobl. bas.	» .....	8.—
	cos.	» .....	28.—
	bas.	» .....	—
	eos.	» .....	3.—
	Erythrobl. bas.	» .....	3.—
	division forms	» .....	—
Leupopoiesis	Megacaryocytes	» .....	0.25
	Mast cells immature	» .....	0.25
	mature.	» .....	—
	Eos. myelocytes	» .....	2.50
	leucocytes band forms	» .....	3.25
	polymorphs	» .....	2.—
	Myeloblasts	» .....	7.75
	Praemyelocytes	» .....	6.50
	Neutro- myelocytes	» .....	20.50
	philes young forms	» .....	32.—
	band forms	» .....	6.50
	polymorphs	» .....	6.50
	Mono- blasts	» .....	6.50
	cytes	» .....	0.25
	Lymphocytes	» .....	3.—
	Plasma & Türk cells	» .....	0.50
	Reticulo endothelial cells	» .....	0.25
	Smear cells	» .....	8.—

Case 42. M. Ø. ♂ aged 69. Born 17—7—1877, Egersund. (Ref. nr. 18947/46).

Occupation: Ex-Headmaster of school.

Prostatectomy in 1934. A couple of years later anaemia was diagnosed

Case 42. Differential cell count of sternal blood.

1946		30—9
Sternal puncture		Asp. easy 0,2 cc. st. blood m.p. + + + + + f.p. + + severe asp. pain
Erythropoiesis	Erythroblasts	27,60
	Leuco-      blasts	
	cytes	72,40
	Promegalomacroblasts	—
	Megalobl.    bas.	—
	eos.	—
	Macrobl.    bas.	3,50
	eos.	—
	Normobl.    bas.	86,—
	eos.	9,—
	Erythrobl.    bas.	—
	eos.	—
	Erythrobl.    bas.	1,—
	division forms eos.	0,50
Leucopoiesis	Megacaryocytes	0,25
	Mast cells    immature	—
	mature	—
	Eos.          myelocytes	1,25
	leucocytes    band forms	2,25
	polymorphs	1,75
	Myeloblasts	2,25
	Praemyelocytes	7,—
	Neutro-      myelocytes	11,75
	philes        young forms	34,75
	band forms	9,50
	polymorphs	12,—
	Mono-        blasts	} 0,50
	cytes	
	Lymphocytes	15,50
	Plasma & Türk cells	1,—
	Smear cells	—
	Reticulo endothelial cells	0,75

and he was given injections. Subsequently he was recommended uninterrupted treatment with liver extract. During the last year signs of a flagging heart. He was admitted to the Seventh Dept. of the Ullevaal Hospital for dyspnoea 11—9—1946.

*Present condition:* Quite severe dyspnoea and slight oedema of ankles and sacral region. Blood press. 210/120. Heart somewhat enlarged. Palpable arteriosclerosis. The usual urine reactions normal. Wassermann  $\div$ . Serum colour 12. S.R. 8 mm. Non-protein nitrogen 78 mg. %. Total serum protein 6.9 %. Globulin 2.5 %, uric acid 14.1 mg. %. Hb. 96 %. Erythrocytes 4.7 mill. Leucocytes 11000, eos. 3 %, bas. 1 %, band forms 2 %, polymorphs 57 %, monocytes 10 %, lymphocytes 27 %.

*Sternal puncture:* Normal morphology-normoblastosis, nucleated blood corpuscles 105000. An electrocardiographic examination showed extrasystole, bundle-branch block, myopathy.

*Case 43. A. Ø. ♀ aged 55. Born 11—4—1881, Aas, Smaalenene. (Ref. nr. 6320/36—37).*

*Occupation:* Wife of farm labourer.

In 1926 (44 years old) she began to suffer from lassitude, loss of appetite, headache and irregularity of menstruation which was intermittent and then ceased. She recovered gradually and was well till April 1927 when she again suffered from lassitude, her skin acquired a yellow tint, and she could not do her work. No soreness of her tongue and no dyspepsia. She felt better in the summer, but late in the autumn she again suffered from lassitude and was breathless at work. Her feet were swollen in the evening, her stools were frequent and loose, and she was very thirsty. For this reason she was admitted to the Med. Dept. B. of the Rikshospital where she stayed from 24—11—1927 to 7—2—1928, being treated for pernicious anaemia.

Case 43. Differential leucocyte count of ear blood.

1937			2—2	18—2	22—2
Praemyelocytes	per cent	.....	2.5	—	1.5
Bas.	»	.....	0.5	0.5	—
Eos.	»	.....	1.0	0.5	2.0
Neutrophils	myelocytes	»	4.5	1.5	2.5
	young forms	»	9.0	1.5	2.0
	band forms	»	6.5	6.0	5.5
	polymorphs	»	41.0	53.5	38.5
Monocytes	»	.....	4.5	2.0	2.5
Lymphocytes	»	.....	30.5	34.5	45.5
Normobl.	bas. in 200 leucocytes	.....	26	—	5
	eos.	»	1	—	1
Macrobl.	bas.	»	3	4	4
	eos.	»	—	—	1
Erythrobl.	bas.	»	9	—	—
	eos.	»	—	—	1



Case 43. Cell count and Hmglb. from ear and sternal blood.

1937	Reticulocytes per mille		Hmglb. per erythrocyte, in %		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
2-2	98	91	46.7	47.2	47	47.—	1.39	1.38	7100	125000
3-2	191									
4-2	173									
5-2	161									
6-2	157		41.7		55		1.82		3500	
8-2	94									
9-2	57		37.1		61		2.27		4800	
11-2	46									
12-2	39		37.8		60		2.19		3000	
13-2	26									
15-2	21									
16-2	19		42.2		53		1.71		3300	
18-2	23	27	44.5	43.4	49	59.—	1.52	1.88	5200	57700
19-2	9									
20-2	39		47.—		50		1.47		4100	
21-2	51									
22-2	81	183	47.8	55.1	53	51.5	1.53	1.29	5700	173600
24-2	218		42.2		52		1.70		4900	
25-2	198									
26-2	189		39.—		54		1.91		5800	
27-2	150									
1-3	20		41.4		48		1.60		4100	
2-4			24.—		85		4.35		6800	

Erythrocytes on admission 1.15 mill. Under liver treatment rise of the reticulocytes to a maximum of 29 %. Erythrocytes on discharge 4.64 mill. As she did not stick to her liver diet, she was admitted for the second time to the Med. Dept. B., staying there from 12-5-1930 to 2-7-1930. Erythrocytes on admission 1.54 mill., on discharge 3.11 mill. For the first year after discharge she stuck to her dietary, taking fully 1 kg. of liver weekly. She felt well, and there were no intervals with relapses. In the middle of October 1936 (3 ½ months ago) she began to suffer from lassitude and a distaste for liver which she had hitherto eaten conscientiously. But from now onwards she faltered in her dietary though she still ate a little liver every day, about ½ kg per week. Bowels now rather loose, but no soreness of tongue or breathlessness. At Christmas 1936 she felt seriously ill, had gradually become pale, was breathless on walking, had little appetite, and believed she had lost weight. Since the middle of January 1937 her appetite has been wretched, she has been unable to eat any liver, and

Case 43. Differential cell count of sternal blood.

1937		2—2	18—2	22—2
Sternal puncture		Asp. easy 2.05 cc. st. blood m.p. +++ f.p. 0 severe a. pain	Asp. easy 0.3 cc. st. blood m.p. + f.p. ++ severe a. pain	Asp. 0.6 cc. st. blood m.p. +++++ f.p. 0 severe a. pain
Erythropoiesis	Erythroblasts per cent	50.3	42.5	52.7
	Leuco- blasts »			
	cytes »	49.7	57.5	47.3
	Promegalomaeroblasts »	1.—	3.5	0.5
	Megalobl. bas. »	1.—	0.5	—
	cos. »	—	—	—
	Macrobl. bas. »	58.7	86.—	49.—
	cos. »	6.7	1.5	2.—
	Normobl. bas. »	15.3	—	44.—
	cos. »	5.—	—	3.—
	Erythrobl. bas. »	4.—	3.—	0.5
	cos. »	6.—	3.—	—
Leucopoiesis	Erythrobl. bas. »	2.3	2.5	1.—
	division forms eos. »	—	—	—
	Megacaryocytes »	—	—	—
	Mast cells immature »	0.3	0.25	0.25
	mature »	—	0.50	—
	Eos. myelocytes »	0.3	0.25	1.—
	leucocytes band forms »	0.3	2.—	1.75
	polymorphs »	1.—	1.50	1.25
	Myeloblasts »	2.—	6.50	3.—
	Praemyelocytes »	2.7	5.75	4.50
	Neutro- myelocytes »	14.3	11.75	12.25
	philes young forms »	26.7	25.50	28.50
	band forms »	10.3	7.—	7.75
	polymorphs »	5.3	12.50	10.—
	Mono- blasts »	} 0.7	1.—	1.25
	cytes »			
	Lymphocytes »	12.7	11.75	9.—
	Plasma & Türk cells »	—	—	—
	Reticulo endothelial cells »	—	—	—
	Smear cells »	23.4	13.75	19.50

could no longer work, taking to her bed 21—1—1937. She now suffered from attacks of shivering, vomiting, nausea, tingling in her fingers. Urine brown as coffee. Temp. 38. A doctor recommended hospital treatment. Out of bed the last three days, but feeling giddy. Appetite somewhat better the last few days, and for three or four days she has eaten a little liver. Admitted to the Med. Dept. A. of the Rikshospital 2—2—1937.

*Present condition:* She is pale and complains of lassitude. Temp. 37.8. Tongue clean, moist, smooth. Resp. unembarrassed. Pulse 80, regular. Blood press. 130/70. Apex beat not definitely palpable. Cardiac dulness 10 cm. to the left of the middle line. A faint blowing murmur over the apex. Liver dulness from 6th rib to 1 fingerbreadth below the costal arch where the margin of the liver is palpable. Slight oedema of the legs. Spleen not palpable. Ewald test meal (after 3/4 hr.) Congo ÷, McLean ÷, ac. 0/5. Urine: Schlesinger (1/10 dilut.) +. S.R. 21 mm. (after 1 hr.). Retics. 98 %<sub>100</sub>. (Regenerative crisis). Serum colour 15. Hb. 47 % = 6.486 g. %. Hb. pr. erythr. 46.7 γγ. Erythrocytes 1.930 mill. Leucocytes 7100. Blood smear: bas. 0.5 %, eos. 0.9 %, praemyelocytes 2.6 %, myelocytes 4.4 %, meta-myelocytes 9.2 %, band forms 5.7 %, polymorphs 41.2 %, monocytes 4.8 %, lymphocytes 30.7 %. Anisocytosis, schizocytosis, microcytosis, poikilocytosis, macro-megalo-cytosis. Polychromasia 5 %<sub>100</sub>. Erythroblasts (normoblasts) 34/200 L. Over-segmentation of the nuclei of the neutrophil cells.

*Sternal puncture:* Macro-normoblastosis. A radiological examination of stomach and duodenum showed nothing abnormal except a long stomach. The patient was involved in a marked regenerative crisis, received no treatment, whereupon the normoblastic regeneration subsided.

*Sternal puncture:* 18—2—1937 macroblastosis retics. 23 %<sub>100</sub>. Erythrocytes 1.52 mill.

*Treatment:* Pernami »Nycos« 20 cc. (19—2).

*Case 44. O. A. ♂ aged 76. Born 10—12—1860, Hedrum. (Ref. nr. 4378/36—37).*

*Occupation:* Ex-handy-man.

Troubled for about 1 ½ years by lassitude which, together with a »wobbly« condition of his legs, has incapacitated him for work. Soreness of his tongue for a year. About a year ago a doctor diagnosed pernicious anaemia and prescribed liver, about 1 kg. weekly. He was admitted to the Med. Dept. A. of the Rikshospital 24—11—1936.

*Present condition:* He is rather thin, suffers from bilateral conjunctivitis. Pulse 76, regular. Resp. unembarrassed. Temp. 37.2. Blood press. 155/90. Pupils normal. Tongue red, perfectly smooth. Liver dulness from 6th rib to 1 finger-breadth above the costal arch. Liver and spleen not palpable. Lower limbs show no visible or measurable atrophy, but both



Case 44. Cell count and Hmglb. from ear and sternal blood.

1936 1937	Reticulocytes per mille		Hmglb. per erythrocyte in $\gamma\gamma$		Hmglb.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
28—11	4	2	44.6	43.—	84.—	67.—	2.60	2.15	4500	5300
29—11	2									
30—11	3	4	41.5	44.2	84.5	66.5	2.81	2.08	6800	12100
1—12	11									
2—12	4		42.1		83.—		2.72		5200	
3—12	3	10	44.7	40.5	84.—	64.—	2.59	2.18	7600	28600
4—12	3									
5—12	45		40.7		80.—		2.71		6000	
6—12	7									
7—12	7	7	40.3	47.3	83.—	71.—	2.84	2.07	8900	28400
8—12	9	4	39.2	34.3	77.—	74.—	2.73	2.97	6700	16300
9—12	9	10	39.2	46.9	82.—	82.5	2.89	2.43	5300	14000
10—12	14	18	37.2	43.4	76.—	69.5	2.81	2.21	7200	9500
11—12	18	22	42.3	43.—	83.—	65.—	2.71	2.09	7400	11600
12—12	31	52	40.—	44.4	80.—	72.5	2.76	2.25	5800	28800
13—12	44									
14—12	32	48	40.2	43.9	89.5	78.—	3.07	2.45	5700	9800
15—12	25									
16—12	11	10	39.7	40.8	90.5	82.5	3.16	2.79	8200	12500
21—12	5									
22—12	7									
24—12	2		37.3		90.—		3.33		7600	
27—12	18		35.6		90.—		3.49		8500	
13—1			33.6		98.—		4.04		8400	

are atactic. Gait atactic. No definite diminution of muscle power. Patellar and Achilles reflexes absent on both sides. Severe pain in hands and feet and marked paraesthesia of his fingers. Buttoning his clothes is difficult. Electrocardiogram normal. Urine: Schlesinger (1/10 dilut.) ++. Ewald test meal (3/4 hr.) ac. 0/5, Congo ÷, McLean ÷. Wassermann ÷. Serum colour 4. S.R. 22 mm. after 1 hr. Hb. 79 % = 10.9 g. %. Hb. pr. erythr. 41  $\gamma\gamma$ . Erythrocytes 2.64 mill. Leucocytes 5300. Retics. 1 %<sub>100</sub>. Blood smear: bas. 0.5 %, eos. 1.5 %, band forms 4.5 %, polymorphs 67.5 %, monocytes 4 %, lymphocytes 20 %. Anicytosis, macrocytosis, slight microcytosis and schizocytosis. Basophil punctuation, polychromasia, orthochromia and over-segmentation of the nuclei of the neutrophil cells.

*Sternal puncture:* Megalo-macroblastosis. Normal conditions found on radiological examination of the stomach and duodenum.

Case 44. Differential cell count of sternal blood.

1936		28—11	30—11	3—12	7—12	8—12	9—12	10—12	11—12	12—12	14—12	16—12
Sternal puncture		Asp. easy 0.45 cc st.-blood m.p. 0 f.p. +++++ no a. pain	Asp. easy 0.3 cc st.-blood m.p. 0 f.p. +++++ no a. pain	Asp. easy 0.35 cc st.-blood m.p. ++ f.p. +++++ no a. pain	Asp. easy 0.6 cc st.-blood m.p. ++ f.p. +++++ no a. pain	Asp. under vac. (20 cc) 0.5 cc st.- blood m.p. traces f.p. +++++ no a. pain	Asp. easy 0.3 cc st.-blood m.p. trac. f.p. +++++ no a. pain	Asp. easy 0.45 cc st.-blood m.p. ++ f.p. +++++ very moderate a. pain	Asp. easy 0.4 cc st.-blood m.p. ++ f.p. +++++ moderate a. pain	Asp. easy 1 cc st.-blood m.p. + f.p. +++++ no a. pain	Asp. easy 0.35 cc st.-blood m.p. ++ f.p. ++ no a. pain	Asp. easy 0.3 cc st.-bl. m.p. traces f.p. +++++ no a. pain
Erythroblasts	per cent	16.—	9.8	14.3	21.5	15.8	19.75	12.6	14.75	37.—	9.75	9.75
Leuco- blasts	“	81.—	90.2	85.7	78.5	84.2	80.25	87.4	85.25	63.—	90.25	90.25
Pronephaloma macroblasts	“	+	0.5	3.—	6.—	4.—	1.—	—	1.—	0.5	—	2.—
Megalobl.	bas.	—	—	1.—	3.—	1.—	1.—	—	—	0.5	—	—
Macrobl.	cos.	14.—	19.5	56.—	17.—	38.—	43.—	27.—	21.—	11.5	8.—	36.—
Normobl.	cos.	9.—	4.—	12.—	8.—	7.—	6.—	8.—	20.—	4.—	—	2.—
cos.	“	45.—	56.—	5.—	19.—	9.—	33.—	37.—	20.—	53.5	64.—	28.—
Erythrobl.	cos.	23.—	18.—	1.—	9.—	28.—	12.—	17.—	18.—	27.—	14.—	10.—
drythrobl.	cos.	5.—	2.—	1.—	2.—	7.—	1.—	9.—	12.—	1.5	14.—	22.—
division forms	cos.	3.—	—	8.—	4.—	5.—	2.—	2.—	4.—	1.5	—	—
Megacaryocytes	“	—	—	—	—	—	—	—	—	—	—	—
Mast cells immature	“	—	—	—	0.75	0.2	—	—	0.25	—	—	0.25
mature	“	—	0.50	—	—	0.2	0.25	0.50	0.25	0.25	0.75	1.—
Eos. myelocytes	“	0.50	—	—	—	0.6	0.25	—	0.25	0.25	0.50	1.—
leucocytes band forms	“	0.50	1.50	2.25	1.50	1.6	0.50	0.25	0.25	1.75	0.25	—
polymorphs	“	1.50	2.75	1.75	1.—	2.6	1.75	0.75	0.50	0.75	1.75	1.75
Myeloblasts	“	1.—	1.—	2.25	2.—	1.2	1.50	1.25	0.75	3.25	6.—	4.50
Pracmyelocytes	“	2.25	1.50	2.75	2.75	1.6	1.75	1.50	1.—	2.—	0.25	0.75
Neutro- myelocytes	“	3.75	3.75	12.—	13.00	10.—	4.—	5.25	5.50	2.50	0.75	0.75
philes young forms	“	8.25	5.25	19.75	26.75	20.4	12.50	11.25	13.75	32.75	6.50	7.25
band forms	“	6.—	4.—	6.—	8.50	9.8	5.25	4.25	4.25	4.75	4.50	9.75
polymorphs	“	59.—	65.50	43.50	32.50	42.2	49.50	55.25	50.—	16.25	52.25	48.—
Mono- blasts	“	4.50	3.—	2.50	1.—	2.2	6.50	7.50	4.50	2.50	7.75	7.75
cytes	“	—	—	—	—	—	—	—	—	—	—	—
Lymphocytes	“	8.25	9.25	3.25	2.—	4.8	7.50	5.50	4.75	10.50	5.75	7.50
Plasma & Turk cells	“	0.25	—	—	0.25	—	—	—	—	0.25	—	—
Reticulo endothelial cells	“	—	—	+	0.25	—	—	—	—	—	—	—
Smear cells	“	4.25	2.—	3.—	6.75	2.4	3.75	1.25	7.50	6.75	3.75	4.75
Cells difficult to diagnose	“	—	—	0.25	—	—	0.25	0.50	—	0.75	—	—

*Treatment:* 28—11—36 10 cc. Ph. Ph. = 500 g. liver.  
 3—12—36 14 cc. Ph. Ph. = 700 g. liver.  
 7—12—36 10 cc. Mrk. E<sup>b</sup> Ph. u. Praec. Bentz E. = 500 g.  
 liver.  
 21/23—12—36 60 cc. Pernami.  
 28/30—12—36 60 cc. Pernami.

*Case 45. P. S. ♂ aged 43. Born 14—10—1894, Toten. (Ref. nr. 8644/37—38).*

*Occupation:* Shopkeeper.

Right leg lame after poliomyelitis in 1911. Confined to bed 1915 for 7 weeks by pleurisy. Digestive disturbances 1925, when a doctor found reduced acidity of the gastric juice. Recovery after dietetic treatment. About 1928 (34 years old) he began to suffer from lassitude and pain in his back, notably between his shoulder-blades. Tingling in arms and legs and considerable soreness of tongue. A doctor consulted in 1930 found haemoglobin 57 %. Pernicious anaemia was diagnosed and liver treatment instituted. Of late years irregular treatment with injections of Pernami, 5 cc. every other to every fourth week. During the last 3 to 4 months 10 cc. Pernami every fortnight. At the same time he ate about 150 g. of liver 2 or 3 times a week. Appetite variable. Constipated. Admitted to the Med. Dept. A. of the Rikshospital 24—3—1938 for pain in abdomen and pain radiating from the small of his back down his legs.

*Present condition:* He looks well. Tongue moist, clean, rather sore. Resp. 16, unembarrassed. Pulse 96, regular. Temp. 37.4. Heart normal, liver and spleen not palpable. Ewald test meal: ac. (after 3/4 hr.) 0/3, Congo ÷, McLean ÷. Urine: Schlesinger (1/10 dilut.) +. Blood press. 140/80. Wassermann ÷. Serum colour 7. (28—3—1938 serum colour 2.5). S.R. after 1 hr. 45 mm. Retics. 6 %<sub>100</sub>. Hb. 85 % = 11.7 g. %. Hb. pr. erythr. 45.2 γγ. Erythrocytes 2.59 mill. Leucocytes 2700. Blood smear: eos. 0 %, band forms 1.9 %, polymorphs 44.8 %, monocytes 5.6 %, lymphocytes 47.7 %.

Case 45. Cell count and Hmglob. from ear and sternal blood.

1938	Reticulocytes per mille		Hmglob.		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
25—3	6	8	85	74.—	2.59	2.41	2700	28200
26—6	10							
27—3	10	21		67.5		2.60		62500
28—3	29	66	87	71.—	3.07	2.15	4400	528400
29—3	60		87		2.96		4300	

Case 45. Differential cell count of sternal blood.

1938		25—3	27—3	28—3
Sternal puncture		Asp. easy 0.35 cc. st. blood m.p. + + + + severe a. pain	Asp. easy 0.4 cc. st. blood m.p. + + + + severe f.p. + + + + a. pain	Asp. under vac. [20 cc.] 0.35 cc. st. blood m.p. + + + + severe f.p. + + + + a. pain
Erythropoiesis	Erythroblasts	25.8	43.8	52.6
	Leuco- blasts			
	cytes	74.2	56.2	47.4
	Promegaloma macroblasts	5.5	1.—	0.25
	Megalobl. bas.	4.5	—	—
	eos.	0.5	—	—
	Macrobl. bas.	50.—	19.5	10.25
	eos.	3.—	0.5	—
	Normobl. bas.	24.5	60.5	77.50
	eos.	8.5	16.—	11.75
	Erythrobl. bas.	0.5	0.5	—
	eos.	1.—	0.5	—
	Erythrobl. bas.	2.—	1.5	0.25
	division forms eos.	—	—	—
Leucopoiesis	Megacaryocytes	+	+	++
	Mast cells immature	—	—	0.25
	mature	0.25	—	0.25
	Eos. myelocytes	1.25	1.—	2.—
	leucocytes band forms	1.25	1.75	2.50
	polymorphs	1.25	2.25	—
	Myelohlasts	5.25	3.25	1.50
	Praemyelocytes	4.—	3.25	7.—
	Neutro- myelocytes	7.—	10.50	18.75
	philes young forms	20.50	19.50	40.50
	band forms	9.25	9.25	11.75
	polymorphs	17.25	12.—	8.25
	Mono- hlasts	1.75	3.50	—
	cytes			
	Lymphocytes	24.—	17.75	2.50
	Plasma & Türk cells	+	0.50	0.50
	Reticulo endothelial cells	0.50	—	0.25
	Sinear cells	6.50	15.—	4.—



Marked anisocytosis, macro-megalocytosis, microcytosis, micro-schizocytosis, polychromasia, slight anisochromia. A few of the neutrophil leucocytes show over-segmentation of their nuclei. A single erythroblast.

*Sternal puncture:* Megalo-macro-normo-blastosis. A radiological examination of stomach, duodenum and large intestine showed normal conditions.

*Treatment:* 25—3—38 40 cc. Pernami prod. nr. 015.

*Case 46. W. B. ♂ aged 72. Born 11—9—1865, Halden. (Ref. nr. 5818/37—38.)*

*Occupation:* Ex-painter.

Well and strong till 70. About a year ago he noticed increasing lassitude, breathlessness and sense of oppression about his heart on exertion. After some weeks he suddenly felt violent pain in both his legs which were tender when touched. A doctor consulted in 1936 started liver treatment. Some time later he received liver injections. No history of dyspeptic symptoms. About 2 months earlier troubled for about 3 weeks by a burning sensation in the left half of his tongue. No loss of weight. Paraesthesias of fingers and feet for about a year. Having become worse latterly, he was admitted to the Med. Dept. A. of the Rikshospital 4—1—1938.

Present condition: He is corpulent and his complexion is rather pale. No complaints of anything in particular. Pulse 84, regular. Temp. 37.7. Tongue moist, clean. Resp. 16, unembarrassed. Blood press. 145/90. Total cardiac dulness 14 cm. A faint systolic murmur. Apex beat not demonstrable. Liver dulness from 5th rib to costal arch, liver not palpable. Reflexes normal. Ewald test meal (after 3/4 hr.) ac. 0/5, Congo ÷, McLean ÷. Urine: Schlesinger (1/10 dilut.) +. Serum colour 8. Wassermann ÷. Retics. 1 %<sub>100</sub>. Blood smear: bas. 3 %, eos. 17 %, band forms 2 %, polymorphs 33 %.

Case 46. Differential leucocyte count of ear blood.

1938		8—1	11—1	12—1	19—1	22—1	15—3
Praemyelocytes	per cent. ....	—	—	—	—	0.5	—
Bas.	" .....	0.5	—	0.5	1.0	1.5	0.5
Eos.	" .....	10.0	10.0	7.5	17.5	7.0	12.0
Neutrophiles	{ myelocytes	" .....	—	—	—	1.5	—
	{ young forms	" .....	—	—	—	—	—
	{ band forms	" .....	2.0	0.5	0.5	1.5	2.0
	{ polymorphs	" .....	41.5	41.5	39.5	30.5	49.5
Monocytes	" .....	1.5	2.0	4.5	2.5	7.0	7.5
Lymphocytes	" .....	44.5	46.0	47.5	48.5	51.0	28.5
Macrobl. bas. in 200 leucocytes		—	—	—	—	—	1

Case 46. Cell count and Hmglob. from ear and sternal blood.

1938	Reticuloocytes per mille		Hmglob. per erythrocyte in $\gamma\gamma$		Erythrocytes in millions		Nucleated blood cells	
	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood	ear blood	sternal blood
4—1	1		67		2.24		3400	
8—1	3	15	74	70	2.25	2.31	4000	129500
9—1	7							
10—1	10							
11—1	4	16	77	79	2.33	2.40	6700	231700
12—1	12	8	77	81	2.31	2.31	7200	116900
13—1	5							
17—1	8							
18—1	5							
19—1	9		72		2.22		6100	
20—1	22							
21—1	86							
22—1	106		75		2.37		7600	
23—1	76							
25—1	66		83		2.57		7000	
27—1	38		85		2.85		9800	
28—1	25							
31—1	24		86		2.97		8000	
3—2	8		93		3.32		8100	
18—2	7		101		3.83		8900	
15—3			100	91	4.12	2.88	7000	204500

monocytes 3 %, lymphocytes 42 %. Marked anisocytosis, macro-megalo-cytosis, schizocytosis, poikilocytosis, microcytosis. Orthochromasia. Polychromasia less than 1 ‰. Slight neutrophil over-segmentation. Hb. 67 % = 9.25 g. %. Hb. pr. erythr. 41.3  $\gamma\gamma$ . Erythrocytes 2.24 mill. Leucocytes 3400.

*Sternal puncture:* Megalo-macroblastosis. Normal conditions found on radiological examination of stomach and duodenum.

*Treatment:* 8/9—1—38 altogether 40 cc. Pernami nr. 0506  
 17/18—1—38       "       4 cc. Pernami forte.  
 22—2—38       "       22 cc. Pernami nr. II.

Case 46. Differential cell count of sternal blood.

1938			8—1	11—1	12—1	15—3
Sternal puncture			Asp. easy 0.75 cc. st. blood m.p. + + + + + f.p. + + moderate a. pain	Asp. easy 0.25 cc. st. blood m.p. + + + + + f.p. + + moderate a. pain	Asp. under vac. [10 cc.] 0.4 cc. st. blood m.p. + + + + + f.p. + + + severe a. pain	Asp. easy 0.15 cc. st. blood m.p. + + + + + f.p. + + moderate a. pain
Erythropoiesis	Erythroblasts	per cent	23.9	14.3	27.4	19.1
	Leuco- blasts	»				
	cytes	»	76.1	85.7	72.6	80.9
	Promegalomacroblasts	»	12.5	9.—	9.—	—
	Megalobl. bas.	»	4.5	—	2.—	—
	eos.	»	—	—	—	—
	Macrobl. bas.	»	59.—	71.—	80.—	6.—
	eos.	»	10.5	5.—	5.—	1.—
	Normobl. bas.	»	6.—	2.—	1.— (+)	52.—
	eos.	»	1.—	—	1.—	41.—
	Erythrobl. bas.	»	1.—	2.—	—	—
	eos.	»	3.—	6.—	—	—
	Erythrobl. bas.	»	2.5	6.—	2.—	—
	division forms eos.	»	—	—	—	—
Leucopoiesis	Megacaryocytes	»	+	+	+	—
	Mast cells immature	»	0.2	0.25	0.50	0.50
	mature	»	—	—	—	0.25
	Eos. myelocytes	»	2.4	1.—	2.75	2.25
	leucoeytes band forms	»	6.2	3.50	7.—	3.75
	polymorphs	»	5.2	3.50	4.50	6.50
	Myeloblasts	»	5.—	3.50	2.25	2.—
	Praemyelocytes	»	5.2	3.75	2.50	8.—
	Neutro- myelocytes	»	16.2	7.75	12.75	6.50
	philes young forms	»	26.—	24.50	31.75	26.25
	band forms	»	9.—	9.—	9.—	13.25
	polymorphs	»	12.6	15.—	15.25	17.50
	Mono- blasts	»	}	0.50	1.—	—
	cytes	»				
	Lymphocytes	»	8.8	17.50	4.25	5.50
	Plasma & Türk cells	»	0.2	0.50	—	0.50
	Reticulo endothelial cells	»	0.8	—	0.50	—
	Smear cells	»	2.2	9.75	6.—	7.25

Case 47. K. K. ♀ aged 70. Born 2—7—1868. (ref. nr. 3847. Med. Dept. Drammen Hospital.

In the spring of 1940 increasing lassitude and pallor. Tinnitus, a sense of hammering in her head, and palpitation of the heart. Has lost about 10 kg. in a year and has felt some soreness of her tongue. Her appetite has been very poor. Admitted to the Med. Dept. of Drammen Hospital 30—1—1941.

*Present condition:* Pale and yellow, jaundiced sclerae. Blood press. 180/80. A systolic blowing murmur over the whole of her heart, loudest over its apex. Liver palpable below the costal arch. Ewald test meal: ac. 0/2, Congo ÷, Serum colour 17. Retics. 13 ‰. Urine: (1/10 dilut) Schlesinger +. S.R. 45 mm. Blood smear: bas. 1 %, eos. 3 %, myelocytes 1 %, metamyelocytes 1 %, polymorphs 53 %, monocytes 1 %, lymphocytes 38 %. Anisocytosis, megalomacrocytosis, microcytosis, poikilocytosis. A few basophilpunctuated erythrocytes. Over-segmentation of the neutrophil leucocytes.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 6—2—41 20 cc. Pernami.

2—2—41 Transfusion of 500 cc. of blood.

Case 47. Cell count and Hinglb. from ear and sternal blood.

1941	Reticulocytes per mille		Hinglb.	Erythrocytes in millions	aspirated sternal blood in cc.	Nucleated blood cells	
	ear-blood	sternal blood				ear-blood	sternal blood
31—1	13	33	20	0.97	0.7	5300	35000
1—2	16						
2—2		33		0.97			
3—2	17	44		1.63			
4—2	24						
5—2	24						
6—2	25	48	25	1.64	0.7		16500
13—2	120	52		1.77			
21—3		81		3.75			

Case 47. Differential cell count of sternal blood.

1941			31—1	2—2	6—2
Erythropoiesis	Erythroblasts	»	33.5	30.8	36.2
	Leuco- blasts	»			
	cytes	»	66.7	69.2	63.8
	Promegalomaeroblasts	»	4.—	8.—	1.5
	Megalobl. bas.	»	5.—	2.5	2.—
	eos.	»	—	—	—
	Macrobl. bas.	»	53.—	52.5	39.—
	eos.	»	23.—	22.5	22.—
	Normobl. bas.	»	—	—	—
	eos.	»	—	—	—
Leucopoiesis	Erythrobl. bas.	»	1.5	1.5	2.5
	eos.	»	9.5	8.—	32.—
	Erythrobl. bas.	»	1.5	4.—	1.—
	division forms eos.	»	2.5	1.—	—
	Megacaryocytes	»	—	0.50	—
	Mast cells immature	»	—	—	0.50
	mature	»	—	0.25	—
	Eos. myelocytes	»	—	—	0.25
	leucocytes band forms	»	—	0.50	0.25
	polymorphs	»	1.75	1.25	2.75
	Myeloblasts	»	2.25	1.—	2.25
	Praemyelocytes	»	1.50	0.50	7.75
	Neutro- myelocytes	»	12.50	4.25	7.75
	philes young forms	»	17.50	6.25	15.50
	band forms	»	7.25	2.75	4.75
	polymorphs	»	27.25	48.25	18.25
	Mono- blasts	»	1.75	2.75	1.—
	cytes	»			
Lymphocytes	»	14.25	22.25	31.75	
Plasma & Türk cells	»	0.50	0.25	—	
Reticulo endothelial cells	»	3.75	3.25	2.25	
Smear cells	»	9.75	6.—	5.—	

*Case 48. A. S. ♀ aged 81. Born 23—10—1858, Modum. (Ref. nr. 3096 Med. Dept. of Drammen Hospital).*

In the autumn of 1939 she began to feel so tired that she could do nothing. She became paler and thinner, and so breathless that she could hardly walk. She was troubled by giddiness, swelling of her legs, and occasionally by a sense of pricking and stabbing in her legs. No soreness of her tongue and no dyspepsia. Admitted to the Med. Dept. of Drammen Hospital 24—1—1940.

*Present condition:* Her skin and mucous membranes are pale, and her sclerae are slightly jaundiced. Blood press. 190/90. Tongue moist, and smooth over its borders. An electrocardiographic examination showed myopathia. S.R. 24 mm. Serum colour 12. Ewald test meal: ac. 0/4, Congo ÷. Urine (1/10 dilut.) Schlesinger +. Hb. 66 %. Erythrocytes 1.59 mill. Leucocytes 4000. Retics. 6‰. Blood smear: eos. 2 %, band forms 2 %, polymorphs 67 %, monocytes 4 %, lymphocytes 25 %. Marked anisocytosis, megalomacrocytosis, schizocytosis, poikilocytosis.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 6—2—40 20 cc. liver extract nr. 62. V.L.T.

Case 48. Cell count and Hmglob. from ear and sternal blood.

1940	Reticulo- cytes per mille	Hmglob.	Erythro- cytes in millions	Nucleated blood cells	
				earblood	sternalblood
25—1	6	66	1.59	4000	
26—1	10				
27—1		58	1.12		105600
28—1	6				
1—2	4	54	1.89	4900	176000
6—2	2	54	1.49	2800	
11—2	100				
12—2	65	58	1.50	5000	
22—2	3	74	2.47		

Case 48. Differential cell count of sternal blood.

1940			27—1	1—2
Sternal puncture			M.p. ++ f.p. + asp. easy 0.5 cc. st. blood	M.p. +++ f.p. + asp. easy 0.4 cc. st. blood
Erythropoiesis	Erythroblasts	per cent	38.8	42.4
	Leuco- blasts	»		
	cytes	»	61.2	57.6
	Promegalomacroblasts	»	13.7	14.7
	Megalobl. bas.	bas.	3.7	8.—
	eos.	»	—	0.3
	Macrobl. bas.	»	54.3	57.—
	eos.	»	20.—	13.7
	Normobl. bas.	»	—	—
	eos.	»	—	—
	Erythrobl. bas.	»	1.—	1.—
	eos.	»	18.7	3.—
	Erythrobl. bas.	»	0.3	0.3
	division forms eos.	»	0.3	2.—
Leucopoiesis	Megacaryocytes	»	—	—
	Mast cells immature	»	0.75	—
	mature	»	—	—
	Eos. myelocytes	»	0.50	0.75
	leucocytes band forms	»	1.—	0.75
	polymorphs	»	0.25	0.50
	Myeloblasts	»	5.50	4.75
	Praemyelocytes	»	4.25	5.25
	Neutro- myelocytes	»	18.25	21.50
	philes young forms	»	26.25	28.75
	band forms	»	9.—	5.—
	polymorphs	»	8.25	12.25
	Mono- blasts	»	}	0.50
	cytes	»		
	Lymphocytes	»	13.75	10.—
	Plasma & Türk cells	»	—	1.25
	Reticulo endothelial cells	»	3.—	2.—
	Smear cells	»	8.75	6.75

*Case 49. H. S. ♀ aged 37. Born 13—8—1902, Ringerike. (Ref. nr. 3438. Med. Dept. Drammen Hospital).*

*Occupation:* Wife of casual labourer.

Late in the autumn of 1939 she began to suffer from increasing tiredness, lassitude and giddiness, with loss of weight and appetite. Admitted to the Med. Dept. of Drammen Hospital 23—1—1940.

*Present condition:* She is thin, asthenic and pale, her skin and sclerae a trifle jaundiced. Tongue smooth. Slight oedema of both legs. Liver definitely palpable 2 finger-breadths below the costal arch. Wassermann ÷. Blood smear: Marked anisocytosis, macronegalocytosis. Poikilo-schizocytosis. Neutrophil over-segmentation of nuclei. Ewald test meal: ac. 7/20, Congo ÷. (8—5—1936: ac. 20/58, Congo +). Serum colour 6. Osm. resistance 0.32—0.52 % NaCl. S.R. 20 mm. Urine: Schlesinger ÷ (1/10 dilut.). Hb. 58 %. Erythrocytes 1.89 mill. Leucocytes 4100. Retics. 0 %.

*Sternal puncture:* Megalo-macroblastosis.

Case 49. Cell count and Hmglob. from ear and sternal blood.

1940	Reticulo-cytes per mille	Hmglob.	Erythro-cytes in millions	Nucleated blood cells	
				earblood	sternalblood
24—1	0	58	1.89	4100	
25—1	4				116400
30—1	6				38000
5—2	50	65	1.76	4200	
6—2	26	61	1.96		
12—2	78				
14—2	29	75	2.52		

*Case 50. B. V. ♀ aged 45. Born 30—11—1894, Krødsherød. (Ref. nr. 3624, Med. Dept. Drammen Hospital.)*

*Occupation:* Wife of labourer.

A year earlier a quite transitory attack of lassitude and tiredness. Well till late in the summer when the same symptoms recurred and were worse, and she suffered at the same time from giddiness and tinnitus, and noticed that her tongue had begun to be sore. She rallied somewhat in the late autumn, but relapsed just before Christmas. Appetite good till a fortnight before admission to hospital. During the last two months loss of weight (10 kg.), and during the past year some stabbing in both her legs and in her fingers in association with numbness. Her hair became rapidly grey during the past winter. Admitted to the Med. Dept. of Drammen Hospital 1—2—1940.



Case 49. Differential cell count of sternal blood.

1940			30—1	25—1
Sternal puncture			Asp. easy 0.3 cc. st. blood m.p. ++ f.p. traces	Asp. easy 0.8 cc. st. blood m.p. ++ f.p. traces
Erythropoiesis	Erythroblasts	per cent	44.5	39.5
	Leuco-        blasts	"		
	cytes	"	55.5	60.5
	Promegalomacroblasts	"	11.5	15.—
	Megalobl.        bas.	"	9.5	7.6
	eos.	"	—	—
	Macrobl.        bas.	"	58.5	44.—
	eos.	"	11.—	27.—
	Normobl.        bas.	"	1.—	—
	eos.	"	—	—
	Erythrobl.        bas.	"	0.5	1.—
	eos.	"	4.5	3.4
	Erythrobl.        bas.	"	3.—	1.7
	division forms eos.	"	0.5	0.3
Leucopoesis	Megacaryocytes	"	—	0.25
	Mast cells        immature	"	0.25	—
	mature	"	—	—
	Eos.            myelocytes	"	2.—	1.75
	leucocytes        band forms	"	1.75	1.25
	polymorphs	"	2.25	1.50
	Myeloblasts	"	3.—	1.50
	Praemyelocytes	"	2.50	3.—
	Neutro-        myelocytes	"	12.50	6.75
	philes        young forms	"	18.75	23.—
	band forms	"	6.75	10.75
	polymorphs	"	11.75	20.25
	Mono-        blasts	"	2.25	0.75
	cytes	"		
	Lymphocytes	"	15.25	14.75
	Plasma & Türk cells	"	0.75	1.—
	Reticulo endothelial cells	"	8.25	9.75
	Smear cells	"	12.—	3.75

*Present condition:* She is thin and pale yellow. Conjunctivae pale, sclerae jaundiced. Tired and finds it difficult to sit up in bed. Tongue smooth. Slight oedema of her legs. Liver dulness from 6th rib to a couple of finger-breadths below the costal arch where definite resistance is demonstrable. Increased liver dulness and palpable resistance under the costal

arch. Electrocardiogram normal. Urine (1/10 dilut.) Schlesinger +. Ewald test meal: ac. 0/3, Congo ÷. Serum colour 10. S.R. 75 mm. Hb. 48 %. Erythrocytes 1.04 mill. Leucocytes 3300. Blood smear: myelocytes 1 %, band forms 6 %, polymorphs 36 %, monocytes 8 %, lymphocytes 49 %. Marked anisocytosis, microcytosis, schizocytosis, poikilocytosis. Many bas. punct. Erythroblasts 1/200 L.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 9—2—40 20 cc. liver extract nr 62 V.T.L.

22—2—40 20 cc. » » » » »

Case 50. Cell count and hmgbl. from ear and sternal blood.

1940	Reticulo- cytes per mille	Hmgbl.	Erythro- cytes in millions	Nucleated blood cells	
				earblood	sternalblood
2—2	20	48	1.04	3300	67000
3—2	28	43	1.05		
7—2	17	34	0.78		158000
9—2	22				
10—2	24	29	0.71		
15—2	85				
22—2	50	58	1.89		
28—2	43	70	2.13		

Case 51. E. J. ♂ aged 52. Born 29—8—1891, Dalen, Asker. (Ref. nr. 11664/42—43).

*Occupation:* Nayvy.

Disease of the glands of the neck at the age of 10, and they have subsequently diminished in size and are now quite small. Inflammation of the cornea 1934 and 1939. A month before admission to hospital he began to suffer from breathlessness on walking up hill and up stairs, and experiencing a sense of oppression in his chest. A little later similar sensations radiating from the inguinal region to his thighs and calves. He gradually became so tired and giddy that he had to discontinue work. Admitted to the Med. Dept. B. of the Rikshospital 31—5—1943.

*Present condition:* He is thin, pale yellow, his sclerae slightly jaundiced. Blood press. 120/85. Pulse 72, regular. Temp. 36.6. Resp. 14, unembarrassed. Tongue moist, not smooth. The slight oedema of his legs passed off in a few days. Many small glands on both sides of his neck along the posterior border of the sterno-cleido-mastoid muscle. A microscopic examination of them showed chronic, specific inflammation, probably tuberculous. Apex beat of heart not demonstrable. Heart sounds clear. Electrocardiogram normal. Spleen palpable 1 finger-breadth below costal arch.

Case 50. Differential cell count of sternal blood.

1940			2—2	6—2
Sternal puncture			Asp. easy 0.4 cc. st. blood	Asp. easy 0.5 cc. st. blood
Erythropoiesis	Erythroblasts	per cent	54.5	59.—
	Leuco- blasts	»		
	cytes	»	45.5	41.—
	Promegalomacroblasts	»	6.—	5.25
	Megalobl. bas.	»	14.5	11.25
	cos.	»	—	—
	Macrobl. bas.	»	50.—	58.25
	cos.	»	20.—	22.25
	Normobl. bas.	»	—	—
	cos.	»	—	—
	Erythrobl. bas.	»	5.—	0.25
	cos.	»	4.—	0.50
	Erythrobl. bas.	»	0.5	1.75
	division forms cos.	»	—	0.50
Leucopoiesis	Megacaryocytes	»	0.25	0.25
	Mast cells immature	»	0.50	0.75
	mature	»	—	—
	Eos. myelocytes	»	1.25	1.50
	leucoocytes band forms	»	3.25	1.75
	polymorphs	»	1.50	0.25
	Myeloblasts	»	1.75	3.50
	Praemyelocytes	»	8.25	0.25
	Neutro- myelocytes	»	18.—	19.25
	philes young forms	»	24.—	26.50
	band forms	»	11.50	13.—
	polymorphs	»	7.50	5.75
	Mono- blasts	»	0.75	0.25
	cytes	»		
	Lymphocytes	»	4.25	4.—
	Plasma & Türk cells	»	1.25	2.—
	Reticulo endothelial cells	»	9.—	4.25
	Smear cells	»	7.—	10.75

Case 51. Differential leucocyte count of ear blood.

1913		2-6	4-6	5-6	6-6	8-6	9-6	10-6	13-6	17-6	23-6	27-6	30-6
Pracmycocytes	per cent.	—	—	—	1.0	2.5	2.0	1.0	0.5	—	—	—	—
Bas.	»	1.5	—	0.5	0.5	—	0.5	0.5	1.0	—	—	—	—
Eos.	»	3.0	0.5	3.0	3.0	2.5	2.5	3.0	2.0	2.0	0.5	0.5	2.0
{ mycocytes young forms band forms	»	—	—	0.5	1.0	1.5	2.5	1.5	—	—	1.5	3.5	1.5
	»	0.5	—	0.5	—	2.5	1.0	—	—	—	—	—	—
	»	1.5	1.0	1.0	2.0	2.0	1.0	1.5	2.0	1.5	3.5	3.0	1.0
Monocytes	»	54.5	67.5	64.0	53.5	62.5	54.0	49.5	64.5	63.5	70.0	59.0	61.0
Lymphocytes	»	4.0	3.5	1.0	2.5	4.5	7.0	6.0	4.0	7.5	4.5	5.5	6.0
Plasma Tlrk	»	34.5	27.5	29.5	36.0	22.0	29.5	37.0	26.0	22.0	20.0	28.0	28.5
Normobl. bas. in 200 leucocytes	»	0.5	—	—	0.5	—	—	—	—	—	—	0.5	—
cos.	—	—	—	—	—	9	1	3	—	—	—	—	—

Case 51. Cell count and Hmglb. from ear and sternal blood.

1943	Reticulocytes per mille		Hmglb.	Erythro- cytes in millions	Nucleated blood cells	
	earblood	sternal blood			earblood	sternal blood
1—6	26		41	1.41	3200	
2—6	21	25	42	1.44	4500	71400
3—6	14					
4—6	25	19	45	1.55	3300	31200
5—6	25		46	1.63	3900	36500
6—6	20	25	47	1.66	5600	36400
7—6	42	34	47	1.65	6300	80900
8—6	171	179	47	1.78	5500	157000
9—6	153		48	1.77	4000	
10—6	131		55	1.86	3700	
11—6	135		57	2.08	2900	
12—6	125					
13—6	111		63	2.18	7800	
14—6	95					
30—6	15	13	79	3.43	9600	

It ceased to be palpable under treatment, and was no longer so on June 21.

Patellar reflexes + + +      + + +  
 Achilles      »      ÷      ÷  
 Plantar      »      VV      VV

A radiological examination of the lungs showed areas of calcification in both lungs and hiluses and along the trachea. Both apices of the lungs retracted. Remains of induration of the parenchyma. Wassermann ÷. S.R. 16 mm. falling to 3 mm. during his stay in hospital. Ewald test meal: ac. 0/5 — histamin-refractory, Congo ÷. Urine: Schlesinger (1/10 dilut.) ÷. Serum albumin 4.77 %. Serum globulin 2.00 %. Total serum protein 6.77 %. Non-protein nitrogen 30.8 mg. %. Uric acid in blood 7.28 mg. %, falling to 6.21 mg. % during his stay in hospital. Cholesterol 120 mg. %. Faeces: sublimate test +. Thromocytes 200,000. Serum colour 17, falling to 4 during his stay in hospital. Retics. 26 ‰. Hb. 41 %. Erythrocytes 1.41 mill. Leucocytes 3200. Blood smear from ear: eos. 3 %, myelocytes 0.5 %, bas. 1 %, metamyelocytes 0.5 %, band forms 1.5 %, polymorphs 54.5 %, monocytes 4 %, lymphocytes 34.5 %, plasma cells 0.5 %. Morphology of the leucocytes normal. Erythrocytes: anisocytosis, microcytosis, schizocytosis, macrocytosis, orthochromia.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 4—6—43 10 cc. Pernami »Nycos« (nr. 149—9—3—43).

5—6—43 »      »      »      »

Case 51. Differential cell count of sternal blood.

1943			2-6	4-6	5-6	6-6	7-6	8-6	30-6
Sternal puncture			Asp. easy 0.1 cc. sternal blood m.p. ++++ f.p. traces severe a. pain	Asp. easy 0.5 cc. st. blood m.p. +++ f.p. 0 moderate a. pain	Asp. easy 0.1 cc. st. blood m.p. + (+) f.p. 0 moderate a. pain	Asp. easy 0.1 cc. st. blood. m.p. + (+) f.p. 0 moderate a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ f.p. 0 severe a. pain	Asp. easy 0.15 cc. st. blood. m.p. ++++ f.p. 0 severe a. pain	Asp. easy 0.4 cc. st. blood m.p. ++++ f.p. 0 moderate a. pain
Erythropoiesis	Erythroblasts	per cent	46.9	35.9	40.5	56.9	72.4	50.1	15.9
	Leuco- blasts	»							
	cytes	»	53.1	64.1	59.5	43.1	27.6	49.9	84.1
	Promegalomacroblasts	»	8.—	6.—	9.5	7.—	1.—	1.—	—
	Megalobl. bas.	»	15.—	8.—	2.—	0.5	—	—	—
	eos.	»	—	—	—	—	—	—	—
	Macrobl. bas.	»	43.—	50.—	60.5	53.5	18.5	12.5	13.—
	eos.	»	8.—	6.5	10.—	3.—	0.5	—	6.—
	Normobl. bas.	»	—	—	—	26.5	53.5	42.5	41.—
	eos.	»	—	—	—	—	24.5	39.—	29.—
Leucopoiesis	Erythrobl. bas.	»	13.—	15.—	5.—	4.—	0.5	2.—	5.—
	eos.	»	10.—	13.—	8.5	4.—	1.—	0.5	1.—
	Erythrobl. bas.	»	2.5	0.5	4.—	1.5	0.5	2.—	3.—
	division forms eos.	»	0.5	1.—	0.5	—	—	0.5	2.—
	Megacaryocytes	»	—	—	0.50	—	—	—	0.50
	Mast cells immature	»	0.50	0.50	0.25	—	0.50	—	0.50
	mature	»	—	0.75	0.25	—	0.50	—	0.25
	Eos. myelocytes	»	1.75	0.25	1.75	1.—	0.25	1.—	0.75
	leucocytes band forms	»	1.—	0.50	0.50	0.25	1.25	0.50	0.25
	polymorphs	»	0.75	1.75	0.75	0.75	0.75	0.75	0.50
	Myeloblasts	»	1.75	0.75	0.75	3.25	0.75	2.—	2.25
	Fraemyelocytes	»	6.—	1.75	3.50	4.75	2.75	3.75	5.—
	Neutro- myelocytes	»	18.50	12.—	15.50	8.—	9.50	12.25	22.—
	philes young forms	»	30.75	17.—	20.25	22.25	16.25	28.—	24.75
	band forms	»	12.50	12.50	12.—	13.25	10.25	14.75	11.75
	polymorphs	»	12.50	20.75	18.50	17.—	20.25	9.75	14.50
	Mono- blasts	»	—	0.75	0.75	0.75	1.50	1.50	0.75
	cytes	»	—	—	—	—	—	—	—
	Lymphocytes	»	7.50	23.—	15.—	15.75	8.25	12.—	9.75
	Plasma & Türk cells	»	0.50	0.75	0.50	—	—	1.—	0.75
	Reticulo endothelial cells	»	1.25	3.—	1.25	1.—	0.50	1.50	0.75
	Smear cells	»	4.75	4.—	8.—	12.—	26.75	10.25	5.—

Case 52. J. B. ♀ aged 63. Born 3—1—1879, Østre Rendal. (Ref. nr. 46; 43—44.)

Occupation: Housekeeper.

In 1931 she began to suffer from lassitude and a tendency to faint. At the same time she became very pale, and at last she could not do her work, and she consulted a doctor who put her on liver treatment, 250 g. daily. On this treatment she recovered quickly. She again suffered from lassitude 1934—1935 and developed pneumonia. She was admitted to hospital and recovered. She relapsed again 1940—1941 when she was given liver injections with good effect. Since then one injection of liver monthly. Late in the spring of 1943 her old symptoms recurred, and she suffered from palpitation of the heart and dyspnoea. During the past half year she has lost weight (10 kg.). She was admitted to the Med. Dept, B, of the Rikshospital 1—7—1943.

*Present condition:* She is pale yellow, and does not complain of pain anywhere. Pulse 84, regular. Blood press. 115/60. Tongue moist, clean, strikingly smooth and furrowed. A soft systolic murmur over the apex. Venous humming over the vessels in the neck. A neurological examination negative. Liver palpable 4 finger-breadths below the costal arch, and the spleen 1—2 finger-breadths below the left costal arch. Electrocardiogram normal. A radiological examination of stomach and duodenum showed no abnormality. Urine: Schlesinger (1/10 dilut.) +. Histamin-resistant. Uric acid 4.69 mg. %. Thrombocytes 74000. Ewald test meal: ac. 0/8. Serum calcium 9.35 mg. %. Serum phosphorus 5.60 mg. %. Choles-

Case 52. Differential leucocytes count of ear blood.

1943		1—7	6—7	10—7	14—7	19—7	21—7	23—7	25—7	31—7	8—8	24—8
Bas. leucocytes	per cent	—	—	—	—	—	0.5	—	—	0.5	1.0	1.75
Eos.	»	5.0	4.0	3.0	3.5	2.5	3.0	2.0	3.0	2.5	7.5	14.00
Neutrophils	myelocytes	—	—	—	—	—	—	1.0	1.0	—	—	—
	young forms	—	0.5	—	—	—	—	1.0	—	—	—	0.50
	band forms	—	5.5	1.0	0.5	0.5	1.5	1.0	—	1.0	1.0	2.50
	polymorphs	46.0	41.5	56.0	49.0	52.5	44.5	35.5	35.0	58.5	58.0	41.00
Monocytes	»	1.5	1.0	1.5	3.5	0.5	5.5	5.0	5.5	3.0	3.5	4.75
Lymphocytes	»	47.5	47.5	38.5	43.5	44.0	45.0	54.0	55.5	34.5	30.0	35.50
Plasma cells	»	—	—	—	—	—	—	0.5	—	—	—	—
Megalobl. bas. in 200 leucocytes		—	1	—	—	—	—	—	—	—	—	—
Macrobl. bas.	»	—	—	—	1	1	7	2	—	—	—	—
eos.	»	—	—	—	—	3	2	4	—	—	—	—
Normobl. bas.	»	—	—	—	—	—	3	—	—	—	—	—
eos.	»	—	—	—	—	—	—	2	—	1	1	—
Erythrobl. cos.	»	—	—	—	2	—	—	—	2	—	—	—

Case 52. Cell count and Hmglob. from ear and sternal blood.

1943	Reticulocytes per mille		Hmglob. per cent	Erythrocytes in millions	Nucleated blood corpuscles		Serum colour
	ear blood	sternal blood			ear blood	sternal blood	
3—7	45	31	34	1.14	3000	110400	13
6—7	17		34	1.36	4800	9200	10
10—7	5		32	1.19	3100		
17—7	3	20	38	1.35	2700	68100	
19—7			33	1.03	3000		15
22—7	100		28	0.84	1600		
23—7	135		33	1.22	3700		
24—7	350						
25—7	138		36	1.62	2200		
27—7	45		41	1.80	3700		
30—7	5		44	1.74	3400	125600	
10—8	84		56	2.15	3800		
28—8	0	3	82	3.83	5100		3

terol 184 mg. %. Serum albumin 3.82 %. Serum globulin 2.61 %. Total serum protein 6.43 %. A/G 1.46. Non-protein nitrogen 27.8 mg %. Takata ÷. Serum colour 13. S.R. 29 mm. Retic. 30 ‰. Hb. 33 %. Erythrocytes 1.42 mill. Leucocytes 3000. Blood smear: eos. 5 %, polymorphs 46 %, lymphocytes 47.5 %; monocytes 1.5 %. The neutrophil leucocytes show over-segmentation. Marked anisocytosis, schizocytosis, microcytosis, polychromasia, orthochromia, macro-megalo-cytosis.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 17—7—43 20 cc. Pernami »Nycos« (nr. 165).  
 18—7—43 20 cc.       »       »       »  
 15—7—43 blood transfusion, 500 cc.  
 5—7—43 20 cc. Pernami (nr. 13515122) given without effect.  
 30/31—7—43 35 cc. Pernami (nr. 115).  
 11/12/13—8—43 altogether 60 cc. Pernami.  
 20/21—8—43 altogether 40 cc. Pernami.



Case 52. Differential cell count of sternal blood.

1943		3—7	6—7	17—7	30—7	28—8
Sternal puncture		Asp. easy 0.2 cc. st. blood m.p. +++ f.p. traces severe a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ f.p. + severe a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ f.p. + severe a. pain	Asp. easy 0.2 cc. st. blood m.p. +++ f.p. + moderate a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ f.p. + severe a. pain
Erythropoiesis	Erythroblasts per cent	54.9	40.1	46.5	43.7	11.4
	Leuco- blasts »					
	cytes »	45.1	69.1	53.5	46.3	88.6
	Promegalomacroblasts »	18.—	6.—	5.—	11.5	—
	Megalobl. bas. »	13.—	5.—	12.—	7.5	—
	eos. »	—	—	—	—	—
	Macrobl. bas. »	39.—	55.—	52.—	51.—	12.—
	eos. »	1.—	7.—	7.—	2.—	4.—
	Normobl. bas. »	—	—	—	0.5	36.—
	eos. »	—	—	—	1.—	42.—
Leucopoiesis	Erythrobl. bas. »	17.5	10.—	9.5	13.5	4.—
	eos. »	10.—	15.—	12.—	11.5	2.—
	Erythrobl. bas. »	1.5	2.—	1.5	1.5	—
	division forms eos. »	—	—	0.5	—	—
	Megacaryocytes »	+	0.50	+	+	0.75
	Mast cells immature »	1.25	0.50	0.50	0.25	0.50
	mature »	—	—	0.25	—	0.50
	Eos. myelocytes »	2.75	2.—	2.25	1.50	2.75
	leucocytes band forms »	4.75	1.5	2.75	2.25	4.—
	polymorphs »	4.75	2.—	4.25	1.75	3.—
	Myeloblasts »	1.75	1.—	2.50	0.75	—
	Praemyelocytes »	7.75	3.5	9.25	10.—	1.75
	Neutro- myelocytes »	9.50	9.—	7.50	8.25	11.—
	philes young forms »	32.50	16.5	23.25	22.75	25.—
	band forms »	8.25	5.—	8.50	7.75	7.25
	polymorphs »	11.50	12.5	9.50	22.—	15.50
	Mono- blasts »	}	—	0.75	2.50	4.—
	cytes »			—	—	—
	Lymphocytes »	7.25	23.5	15.75	9.75	20.—
	Plasma & Türk cells »	—	2.—	1.75	—	0.25
	Smear cells »	5.25	19.—	6.75	7.25	2.25
	Reticulo endothelial cells »	2.75	1.5	3.50	3.75	1.50

Case 53. J. K. ♂ aged 73. Born 13—8—1873, Ullensaker. (Ref. nr. 8737/46).

Occupation: Ex-cutter-out.

About Christmas 1939 progressive lassitude, loss of appetite and weight, swelling of the ankles and increasing breathlessness were observed. His skin turned pale yellow, and he was subject to attacks of vomiting. Admitted to the 8th Dept. of Ullevaal Hospital with the diagnosis of pernicious anaemia 1—6—40. Here the haemoglobin was 40 %, and the erythrocytes numbered 1.9 mill. Ewald test meal: ac. 0/6, Congo ÷. Electrocardiogram normal. Wassermann ÷. Small haemorrhages of the retina. A radiological examination of stomach and duodenum showed normal conditions. Benzi-din test of the faeces ÷. Under liver treatment the haemoglobin rose to 94 %, and the number of erythrocytes 4.5 mill.

Re-admitted 3—3—44 to Ullevaal Hospital, this time to the 7th Dept. because of the inadequacy of the liver treatment. In addition to his old troubles, he now complained of his fingers being cold and of cramp in his legs. Hb. 70 %, erythrocytes 2.92 mill. Under liver treatment these figures rose respectively to 92 % and 4.73 mill. Normal electrocardiographic findings. A neurological examination showed slight signs of funicular myelosis. A radiological examination of stomach and duodenum was still negative.

He was again admitted to the 7th Dept. of Ullevaal Hospital 25—4—46.

Case 53. Cell count and haemoglobin from ear and sternal blood.

1946	Reticulo-cytes per mille	Hmglob. per cent	Erythro-cytes in millions	Nucleated cells		Asp. quantity sternal blood cc.
				ear blood	sternal blood	
30—4	4	48	1.50	4100	76700	0.4
6—5	10	40	1.60	3300		
9—5	2					
11—5	2	31	1.15	1400	140500	0.3
13—5	4	32	1.16	2800	138800	0.3
14—5	30					
16—5	90	30	1.29	9900	412500	0.3
17—5	400					
18—5	220	40	1.23	2100	84000	0.4
20—5	106		1.64	5900		
24—5	46	54	1.78	4500		
29—5	2	57	1.90	8500		
3—6	116	58	2.16	5500		
2—7		82	3.13	4000		

Serum colour: 14/5 7, 20/5 8, 23/5 6, 27/5 6, 10/6 5.

Case 53. Differential cell count of sternal blood.

1946		2—5	11—5	13—5	16—5	18—5
Sternal puncture		Asp. easy 0.4 cc. st. blood m.p. + + + + f.p. + + + severe a. pain	Asp. easy 0.3 cc. st. blood m.p. + + + + f.p. + + + severe a. pain	Asp. easy 0.3 cc. st. blood m.p. + + + + f.p. + + + moderate a. pain	Asp. easy 0.2 cc. st. blood m.p. + + + + f.p. + + + severe a. pain	Asp. easy 0.4 cc. st. blood m.p. + + + + f.p. + + +
Erythropoiesis	Erythroblasts per cent	49.90	35.—	49.80	56.60	38.30
	Leuco- blasts »					
	cytes »	50.10	65.—	50.20	43.70	41.70
	Promegalomacroblasts »	7.50	3.—	4.—	0.75	1.—
	Megalobl. bas. »	17.—	3.50	8.50	—	—
	eos. »	—	—	—	—	—
	Macrobl. bas. »	30.—	13.—	19.—	20.—	11.50
	eos. »	35.—	71.50	61.50	—	1.50
	Normobl. bas. »	—	—	—	30.75	30.—
	eos. »	—	—	—	43.25	52.—
Leucopoiesis	Erythrobl. bas. »	—	5.—	0.50	2.25	1.—
	eos. »	9.—	3.50	3.50	0.50	1.50
	Erythrobl. bas. »	0.50	0.50	2.—	1.25	1.50
	division forms eos. »	1.—	—	0.50	1.25	—
	Megacaryocytes »	—	—	—	0.75	—
	Mast cells immature »	0.75	—	0.25	—	0.50
	mature »	—	—	—	—	—
	Eos. myelocytes »	3.25	2.25	3.75	3.50	1.—
	leucocytes band forms »	3.50	1.75	3.50	1.—	3.75
	polymorphs »	1.50	3.25	2.25	0.25	2.50
	Myeloblasts »	2.—	3.50	6.50	3.50	2.75
	Pracmyelocytes »	8.25	8.25	9.—	4.50	3.25
	Neutro- myelocytes »	13.25	7.—	12.25	19.75	12.50
	philes young forms »	31.—	17.75	25.75	36.75	30.50
	band forms »	5.—	4.—	3.25	3.25	7.50
	polymorphs »	2.25	3.—	2.75	2.50	5.25
	Mono- blasts »	} 1.25	1.50	2.—	1.—	1.50
	cytes »					
	Lymphocytes »	13.50	38.25	20.50	7.75	18.—
	Plasma & Türk cells »	0.50	1.50	1.75	0.50	1.50
	Smear cells »	9.25	6.—	5.—	10.50	9.25
	Reticulo endothelial cells »	4.75	2.—	1.50	4.50	0.25

for the same troubles as before and again because he had neglected his liver treatment. Skin and sclerae now somewhat jaundiced. Blood press, 170/95, falling to 130/70. Electrocardiogram normal. S.R. 33—60 mm. falling to 6 mm. on his discharge. Chlorides 140 m. equiv./l. Non-protein nitrogen 52 mg. %. Blood platelets 260,000. Takata's reaction  $\div$ . Blood group A. Wassermann  $\div$ . Meinicke reaction  $\div$ . Ewald test meal: Congo  $\div$ . Coagulation time 9 to 15 min. (Gram's coagulometer). Total proteins in serum 6.1 %, alb. 3.8 %, glob. 2.3 %. Calcium 9.9 mg. %. Ph. 3.4 mg. %. Total bases 153.7 m. equiv./l. Blood smear eos. 3 %, band form 8 %, polymorphs 47 %, lymphocytes 40 %, monocytes 2 %. Marked anisocytosis, macro (megalo)-cytosis, schizocytosis, poikilocytosis, microcytosis, orthochromia.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 8—5—46 20 cc. Pernami «Nycos» nr. 194.

13—5—46 55 cc.       "       "       "

27—5—46 40 cc.       "       "       nr. 198.

*Case 54. C. H. ♂ aged 71. Born 21—8—1875, Urskog. (Ref. nr. 13203/46).*

*Occupation:* Ex-agent.

In 1930 confined to bed for 6 months by anaemia. Complete recovery under parenteral liver treatment. Subsequently well on a dietary of liver, but when it was difficult to obtain in 1940, he took to injection treatment. In 1942 operated on, cancer of the stomach being suspected, but his symptoms were found to be due to a foreign body in the stomach. In 1943 he became worse, being troubled by progressive lassitude and coldness and «withering» of his lower limbs. Admitted to the Lovisenberg Hospital where he was treated with blood transfusion and liver injections. Ewald test meal said to have given normal acid figures. In August 1943 he was admitted to the 8th Dept. of Ullevaal Hospital where his haemoglobin was 42 % and erythrocytes numbered 1.75 mill. Ewald test meal: ac. 0/8. Serum colour 2. Blood press. 100/70. Electrocardiogram normal. A radiological examination of stomach and duodenum showed nothing abnormal apart from post-operative deformity of bulbus duodeni. Liver treatment with good effect. In 1945 treated at the 2nd Dept. of Ullevaal Hospital for tuberculous osteomyelitis. Discharged 29—10—1945 with a sinus in his right thigh.

Much troubled after discharge from hospital by pain in both legs, growing lassitude and dysphagia. Liver treatment very irregular. He was therefore admitted to the 7th Dept. of Ullevaal Hospital 28—6—1946 when it was noted that there was still a discharging sinus in his right thigh. Blood press. 140/70. Tongue smooth. Spleen and liver not palpable. No definite enlargement of the lymphatic glands. Achilles reflexes  $\div$ , a neurological examination being otherwise negative. Urine: Schlesinger +

Case 54. Cell count and haemoglobin from ear and sternal blood.

1946	Reticulo- cytes per mille	Hmglob. per cent	Erythro- cytes in millions	Nucleated cells		Asp. quantity sternal blood cc.
				ear blood	sternal blood	
29—6	2	31	1.03	1700		
2—7	8	27	0.89	1900		
3—7	12				96000	0.3
4—7	10	29				
5—7		27	0.88		239000	0.6
6—7	8					
8—7	8	27	0.75			
9—7	150				128000	
10—7	184	33	1.13			
11—7	182					
12—7	52	34	1.48	4800		
15—7	10	43	1.65	6500		
19—7		41	1.73	6100		
22—7		38	1.76	6000		

( $\frac{1}{10}$ ) albumin +. Wassermann ÷. Benzidin test of faeces ÷. Non-protein nitrogen 30 mg. %. Serum colour 3.5 and 1. S. R. 100 to 19 mm. Osmotic resistance: commencing haemolysis at 0.44 % NaCl and total haemolysis at 0.30 % NaCl. 2/7. Total serum protein 5 %, alb. 3.2 %, glob. 1.8 %. Total bases 150 m. equiv./l. Ph. 2.7 mg. %. Calcium 8.8 mg. %. Radiological examination of stomach, duodenum and *oesophagus*: negative, of chest, remains of pulmonary infiltration on the right side and bilateral disease of the pleura. An osteomyelitic defect of the right leg. Blood smear from the ear: eos. 6 %, bas. 1 %, band forms 1 %, polymorphs 53 %, lymphocytes 38 %, monocytes 1 %. Marked anisocytosis, macro-megalo-cytosis, micro-schizocytosis, poikilocytosis. Marked over-segmentation of the leucocytes.

*Sternal puncture*: Megalo-macroblastosis.

*Treatment*: 4—7—46 20 cc. Pernami «Nycos» nr. 209.

5—7—46 20 cc. Pernami «Nycos» nr. 209.

Case 54. Differential cell count of sternal blood.

1946		3—7	5—7	9—7
Sternal puncture		Asp. easy 0.3 cc. st. blood m.p. +++++ f.p. 0 severe a. pain		
Erythropoiesis	Erythroblasts	39.8	27.3	63.7
	Leuco blasts			
	cytes	60.2	72.7	36.3
	Promegalomaeroblasts	3.—	5.5	2.—
	Megalobl. bas.	10.—	12.—	—
	eos.	—	—	—
	Macrobl. bas.	59.5	57.—	9.—
	eos.	3.—	18.5	—
	Normobl. bas.	—	—	77.5
	eos.	—	—	8.5
	Erythrobl. bas.	12.5	0.5	—
	eos.	10.5	4.—	—
Leucopoiesis	Erythrobl. bas.	1.5	1.—	3.—
	division forms eos.	—	1.5	—
	Megacaryocytes	0.25	+	0.25
	Mast cells immature	0.25	—	0.75
	mature	—	—	0.25
	Eos. myelocytes	0.75	1.—	2.—
	leucocytes band forms	0.50	1.—	1.—
	polymorphs	0.50	1.—	0.75
	Myeloblasts	1.75	0.50	1.—
	Praemyelocytes	7.50	11.75	17.50
	Neutro- myelocytes	16.25	14.25	17.25
	philes young forms	50.75	33.50	37.50
	band forms	8.50	3.75	7.25
	polymorphs	6.25	6.75	8.75
	Mono- blasts	}	0.25	—
	cytes			
	Lymphocytes	1.—	17.75	1.50
	Plasma & Türk cells	1.25	1.50	0.75
	Smear cells	3.25	6.25	1.25
	Reticulo endothelial cells	1.25	0.75	2.25

Case 55. M. A. ♀ aged 82. Born 1—1—1864, Sanne. (Ref. nr. 12037/46).

Occupation: Widow.

In 1932 admitted to the 7th Dept. of Ullevaal Hospital after having suffered for some time from dyspeptic disturbances and progressive pallor and lassitude. Pernicious anaemia was diagnosed, hb. 44 %. Good effects from liver treatment. Thereafter several periods in hospital because she neglected liver treatment. Last admission 12—6—1946.

*Present condition:* Severe pain in the upper abdomen traced to disease of the biliary passages. She was tired, exhausted, thin. Temp. 37.2. Arteries rigid. Pyuria. Wassermann +. Serum protein 6.3 %, alb. 3.8 %, glob. 2.5 %. S.R. 120 and 126 mm. Nonprotein nitrogen 30 mg. %. Blood smear: eos. 8 %, bas. 2 %, band forms 7 %, polymorphs 40 %, lymphocytes 40 % monocytes 3 %. Marked anisocytosis, microcytosis, macro-(megalo) cytosis. Orthochromia, schizocytosis, poikilocytosis. Morphology of the leucocytes normal. An electrocardiographic examination showed myopathy. 1945 blood press. 225/100.

*Sternal puncture:* Megalo-macroblastosis.

While in hospital she had high fever (38—39). Progressive lassitude and drowsiness, and she died 7—7—1946. Death was traced to senile debility with general arteriosclerosis, and disease of the urinary and biliary tracts. Normal reaction of the bone marrow to liver treatment. A post-mortem examination showed general arteriosclerosis, gall-stones, light red marrow in the vertebral column, and diffuse pigmentation of the liver cells with iron.

*Treatment:* 17—6—46 20 cc. Pernami «Nycos» nr. 209.

Case 55. Cell count and haemoglobin from ear and sternal blood.

1946	Reticulo- cytes per mille	Hmglob. per cent	Erythro- cytes in millions	Nucleated cells		Serum colour
				ear blood	sternal blood	
13—6						
17—6	4	49	2.01	2900	76000	11
18—6	10					
19—6	70	40	1.56			
20—6	190	38	1.57	2100	900000	5
21—6	226	45				
22—6	196	47	1.85			
23—6	136					
24—6	86	46	1.85	8000		
26—6	37	48	1.98	13500		
28—6	22	48	1.89			
1—7	24	52	2.06	6500		
5—7		55	2.14			4

Cases 55. Differential cell count of sternal blood.

1946			13—6	20—6
Sternal puncture			Asp. easy 0.3 cc. st. blood m.p. +++ f.p. ++ severe a. pain	Asp. easy 0.1 cc. st. blood m.p. +++ f.p. + severe a. pain
Erythropoiesis	Erythroblasts	per cent	33.30	66.60
	Leuco-        blasts	"		
	cytes	"	66.70	33.40
	Promegalomaeroblasts	"	4.—	4.—
	Megalobl.        bas.	0a	13.50	—
	eos.	"	—	—
	Macrobl.        bas.	"	51.—	17.50
	eos.	"	12.—	—
	Normobl.        bas.	"	—	67.—
	eos.	"	—	7.50
	Erythrobl.        bas.	"	11.50	—
	eos.	"	7.50	—
	Erythrobl.        bas.	"	0.50	4.—
	division forms    eos.	"	—	—
Leucopoiesis	Megaerythrocytes	"	—	—
	Mast cells        immature	"	0.75	0.25
	mature	"	—	0.25
	Eos.               myelocytes	"	1.75	1.25
	leucocytes        band forms	"	1.75	0.75
	polymorphs	"	1.75	1.—
	Myeloblasts	"	2.25	0.75
	Praemyelocytes	"	4.75	10.75
	Neutro-        myelocytes	"	11.25	19.50
	philes        young forms	"	26.50	13.25
	band forms	"	6.—	1.50
	polymorphs	"	11.25	4.—
	Mono-        blasts	"	—	—
	cytes	"	—	—
	Lymphocytes	"	18.25	2.25
	Plasma & Türk cells	"	0.25	1.50
	Smear cells	"	12.75	11.25
	Reticulo endothelial cells	"	0.75	1.50



Case 56 M. F. ♂ aged 68. Born 21—8—1878, Oslo. (Ref. nr. 19828/45).

Occupation: Civil servant.

Pulmonary tuberculosis in his youth. In 1941 began to suffer from soreness of mouth and tongue and increasing lassitude and pallor. Pernicious anaemia diagnosed in 1943. Liver treatment said to have been ineffective. As he continued to suffer from increasing dyspepsia and lassitude, his skin becoming jaundiced, he was admitted to the VIIth Dept. of Ullevaal Hospital 23—10—1945.

*Present condition:* Complaints of lassitude. Skin pale yellow, tongue smooth and atrophic. Blood press. 125/70. No palpable, pathological enlargement of the lymphatic glands. Liver and spleen not enlarged. Temp. 36. Urine: Schlesinger (1/10) +. S.R. 24 to 4 mm. Cholesterol 200 mg. %<sub>100</sub> 24—10—1945. Total serum protein 7 %, alb. 4.8 %, glob. 2.2 %. Total bases 153 m. equiv./l. Serum iron 242 gamma %. Electrocardiogram normal. Radiological examination of stomach and duodenum normal. Blood smear from ear: eos. 1 %, bas. 0 %, band forms 6 %, polymorphs 52 %, lymphocytes 38 %, monocytes 3 %. Macromegalo-cytosis, micro-poikilo-schizocytosis. Basophil punctuation, orthochromia. A few macroblasts.

*Sternal puncture:* Macro-megaloblastosis.

*Treatment:* 25—10—45 40 cc. Pernami »Nycø» nr. 181.

21—11—45 35 cc. Pernami »Nycø» nr. 181.

Case 56. Cell count and Haemoglobin from ear and sternal blood.

1946	Reticulo-cytes per mille	Hmglb. per cent	Erythro-cytes in millions	Nucleated cells		Serum colour	Aspir. quantity sternal blood cc.
				ear blood	sternal blood		
24—10	0	34	0.94	2700		20	
25—10	0				137800		0.3
26—10	7	36	1.03	2900	225600		0.3
27—10	80	33	1.10	2500	127200		0.3
28—10	110						
29—10	180	36	1.12	2900	238800	15	0.3
30—10	200	41	1.30				0.6
31—10	210	41	1.30				
1—11	140						
2—11	84						
3—11	54	44	1.49				
5—11	20	47	1.70			6	
7—11	0	55	1.90				
10—11	3	50	1.92	7800	120000		0.4
15—11	5	58	1.86	8200			
21—11	5	55	1.93	8600			
15—12		70	3.05				

Case 56. Differential cell count of sternal blood.

1946			25—10	26—10	27—10	28—10	29—10	30—10	10—11
Sternal puncture			Asp. easy 0.3 cc. st. blood m.p. + + + f.p. + severe a. pain	Asp. easy 0.3 cc. st. blood m.p. + + + + f.p. + severe a. pain	Asp. easy 0.3 cc. st. blood m.g. + + + + f.p. + severe a. pain		Asp. easy 0.25 cc. st. blood m.p. + + + + f.p. + moderate a. pain	Asp. easy 0.6 cc. st. blood m.p. + + + + f.p. + moderate a. pain	Asp. easy 0.4 cc. st. blood m.p. + + + + f.p. + moderate a. pain
Erythropoiesis	Erythroblasts	per cent	54.80	56.—	61.50	75.80	78.60	70.90	19.50
	Leuco- blasts	"							
	cytes	"	15.20	44.—	48.50	24.20	21.40	29.10	80.50
	Promegalomacroblasts	"	18.—	7.—	8.50	2.—	3.50	3.—	11.—
	Megalobl. bas.	"	2.—	0.50	1.—	0.50	—	—	—
	eos.	"	2.50	—	—	—	—	—	—
	Macrobl. bas.	"	15.—	52.50	53.50	32.50	20.—	9.50	35.—
	eos.	"	39.—	4.50	34.50	4.50	3.—	—	0.50
	Normobl. bas.	"	—	—	1.50	40.—	25.50	78.50	47.—
	eos.	"	—	—	—	16.—	46.50	7.—	3.50
	Erythrobl. bas.	"	5.50	14.50	—	0.50	—	—	0.50
	eos.	"	16.50	19.—	1.—	0.50	—	—	0.50
Leucopoiesis	Erythrobl. bas.	"	1.—	2.—	1.—	1.50	0.50	2.—	2.—
	division forms eos.	"	1.—	—	—	2.—	1.—	—	—
	Megacaryocytes	"	0.25	—	0.50	—	—	—	—
	Mast cells immature	"	—	0.25	0.25	—	0.50	0.25	—
	mature	"	—	—	—	—	—	0.25	0.50
	Eos. myelocytes	"	1.—	1.50	0.75	2.75	2.75	1.50	0.75
	leucocytes band forms	"	2.—	0.50	1.25	1.—	1.—	1.50	0.50
	polymorphs	"	0.25	1.—	0.25	0.25	0.25	1.—	1.—
	Myeloblasts	"	2.—	0.25	1.—	8.—	6.25	1.75	3.25
	Praemyelocytes	"	11.—	7.—	13.—	9.—	8.75	13.—	6.50
	Neutro- myelocytes	"	16.50	24.25	21.50	19.25	17.50	15.25	15.—
	philes young forms	"	36.75	47.75	41.—	33.75	32.25	45.75	26.25
	band forms	"	6.50	8.50	1.75	3.75	8.50	2.—	10.75
	polymorphs	"	5.—	6.75	2.50	5.75	6.50	2.25	21.50
	Mono- blasts	"	}	—	—	1.—	1.50	—	1.—
	cytes	"				—	—	—	—
	Lymphocytes	"	3.75	1.25	3.—	6.75	6.50	5.75	8.50
	Plasma & Türk	"	2.75	—	0.75	2.75	0.75	1.50	—
	Smear cells	"	9.25	2.75	10.—	3.75	6.—	6.50	2.75
	Reticulo endothelial cells	"	3.50	1.25	2.50	3.25	1.50	1.75	1.75

Case 57 A. W. B. ♂ aged 62. Born 11—10—1884, Stockholm. (Ref. nr. 6349/46).

Occupation: Professor.

Very fat for many years. Operated on in 1942 for intestinal obstruction. Signs of hypertrophy of the prostate for several years. Troubled during the last few months by progressive lassitude, breathlessness and loss of appetite. Nothing in his history to suggest funicular myelosis. No soreness of his tongue. Admitted to the Surg. Dept. of Ullevaal Hospital 22—3—1946, being subsequently transferred to the 7th Dept. with the diagnosis of pernicious anaemia.

*Present condition:* Great lassitude, but perfectly conscious. Pulse 100, regular. Blood press. 125/80. Tongue moist and clean, not smooth. No enlargement of the lymphatic glands. Spleen and liver not palpable. Normal findings on a neurological examination. S.R. 49 mm. Serum colour 2. Total serum proteins 7.3 %, alb. 5.1 %, glob. 2.2 %. Ca. 10.3 mg %, phos. 2.3 mg. %. Total bases in serum 148 m. equiv./l. Osmotic resistance: commencing haemolysis at 0.54 % NaCl. and total haemolysis at 0.34 %. Coagulation-time normal. 25—3—1946 blood platelets 97,000 and 3—4—1946, 590,000. Electrocardiogram normal. Urine: Schlesinger (1/10) ÷. Ewald test meal: (1942) ac. 50/70 and (1946) 0/3.

*Treatment:* 29—3—46 40 cc. Pernami »Nyco» nr. 194.

Case 57. Cell count and Haemoglobin from ear and sternal blood.

1946	Reticulo- cytes per mille	Hmglob. per cent	Erythro- cytes in millions	Nucleated cells	
				ear blood	sternal blood
25—3	0	49	1.40	2700	93200
27—3	2	49	1.28		
29—3	35	46	1.35		35100
30—3	55				
31—3	96	58	1.60		253500
1—4	116			7900	
2—4	110	58	1.62		
3—4	80				
4—4	35	58	1.88		
6—4	30	62	1.99		
8—4	37	60	1.96		
10—4	35	65	1.97		
12—4	15	65			141000

Case 57. Differential cell count of sternal blood.

1946		25—3	30—3	1—4	12—4
Sternal puncture		Asp. easy 0.6 cc. st. blood m.p. + + + + + f.p. traces severe a. pain	Asp. easy 0.4 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. easy 0.22 cc. st. blood m.p. + + + + + f.p. traces severe a. pain	Asp. easy 0.2 cc. st. blood m.p. + + + + + f.p. + + moderate a. pain
Erythropoiesis	Erythroblasts	41.70	44.60	48.80	40.80
	Leuco-        blasts				
	cytes	58.30	55.40	51.20	59.20
	Promegalomacobl.	6.—	1.50	8.—	2.50
	Megalobl.    bas.	16.50	3.50	—	—
	eos.	—	—	—	—
	Macrobl.    bas.	59.—	87.50	31.—	11.—
	eos.	8.—	—	4.50	—
	Normobl.    bas.	—	1.50	27.—	29.50
	eos.	—	—	26.50	55.50
	Erythrobl.    bas.	3.50	1.—	—	—
	eos.	4.—	—	0.50	—
	Erythrobl.    bas.	1.—	2.—	1.50	1.—
	division forms	2.—	—	1.—	0.50
Leucopoiesis	Megacaryocytes	—	—	0.25	—
	Mast cells    immature	0.50	0.25	—	—
	mature	—	—	—	—
	Eos.        myelocytes	4.25	2.—	2.25	3.25
	leucocytes    band forms	1.75	0.75	1.—	2.—
	polymorphs	2.25	1.25	0.50	1.25
	Myeloblasts	1.25	1.25	1.25	1.75
	Praemyelocytes	3.—	9.50	3.25	2.75
	Neutro-    myelocytes	32.25	23.50	21.75	25.50
	philes        young forms	37.—	10.25	46.25	40.—
	band forms	4.75	2.75	8.75	6.50
	polymorphs	2.50	2.50	7.25	8.50
	Mono-        blasts	0.25	—	—	—
	cytes				
	Lymphocytes	2.50	0.50	0.75	1.50
	Plasma & Türk cells	0.75	—	—	—
	Smear cells	6.—	13.75	6.50	5.75
	Reticulo endothelial cells	1.—	1.75	0.25	1.25

Case 58. A. B. R. ♂ aged 70. Born 15—1—1876, Krödsherrad. (Ref. nr. 5082/45).

Occupation: Ex-carpenter.

Since 1933 treated for high blood pressure and angina pectoris, and since 1940 for diabetes mellitus. Troubled by eczema for many years. Since the beginning of 1945 progressive jaundice and dark urine. Poor appetite for a spell, but otherwise he feels quite well. Admitted for jaundice to the 7th Dept. of Ullevaal Hospital 12—3—1946.

*Present condition:* Skin and sclerae jaundiced. Blood press. 155/65. Tongue rather smooth along its margins. Patellar and Achilles reflexes ÷. Sensation somewhat reduced in hands and feet. Temp. 37.7. Electrocardiogram normal. Radiological examination of stomach and duodenum negative. Heart enlarged and aortaformed. Coagulation-time 10 to 15 minutes (Gram's coagulometer). 13—3—1946, serum proteins 6 %, alb. 3.7 %, glob. 2.3 %. Thrombocytes 293,000. Takata ÷, cholesterol 133 mg. %. S.R. on admission 21 mm., rising to 55 mm. (14—4—1946) and sinking to 7 mm. on his discharge from hospital. Ewald test meal: ac. 0/1 Benzidin test of faeces ÷. Wassermann ÷. Uric acid 9.3 mg %, on discharge 6 mg. %. Blood smear from ear: eos. 2.5 %, bas. 1.5 %, band forms 2 %, poly-

Case 58. Cell count and Haemoglobin from ear and sternal blood.

1946	Reticulo- cytes per mille	Hmgbl. per cent	Erythro- cytes in millions	Nucleated cell		Serum colour
				ear blood	sternal blood	
13—3		34	1.43	2400	57900	9
15—3	2					
19—3	0					14
21—3		34	1.43	2600		
24—3	10	32	1.32	2800	62800	15
25—3						
26—3	16					
27—3	110					
28—3	142					
29—3						
30—3	130	44	2.02	3600		9
3—4	80					
4—4	70	46	2.05	5000		
6—4	20	47	2.28	4800		
12—4		51	2.49	4100		
17—4		60	3.04	4600		5
25—5		71	3.58	4200		
21—6		86	3.39	4700	37300	10

Case 58. Differential cell count of sternal blood.

1945			15—3	24—3	26—3	20—6
Sternal puncture:			Asp. easy 0.3 cc. st. blood m.p. + + + + + f.p. 0 moderate a. pain	Asp. easy 0.1 cc. st. blood m.p. + + + + + f.p. + + moderate a. pain	Asp. easy 0.2 cc. st. blood m.p. + f.p. + moderate a. pain	Asp. easy 0.3 cc. st. blood m.p. + + + + + f.p. + + moderate a. pain
Erythropoiesis	Erythroblasts	per cent	53.9	43.5	65.8	35.1
	Leuco- blasts	»				
	cytes	»	46.1	46.5	34.2	64.9
	Promegalomacroblasts	»	11.—	2.5	2.—	3.—
	Megalobl. bas.	»	23.5	6.—	—	1.5
	cos.	»	—	—	—	—
	Macrobl. bas.	»	44.5	58.5	9.5	22.5
	cos.	»	14.5	15.—	2.5	7.—
	Normobl. bas.	»	—	—	49.—	24.5
	cos.	»	—	—	34.—	40.—
	Erythrobl. bas.	»	1.5	8.5	—	—
	cos.	»	2.5	9.—	—	—
	Erythrobl. division forms	»	1.—	0.5	1.—	1.—
	cos.	»	1.5	—	2.—	0.5
Leucopoiesis	Megacaryocytes	»	+	+	0.25	+
	Mast cells immature	»	0.25	—	—	—
	mature	»	0.25	—	—	0.25
	Eos. myelocytes	»	1.25	1.25	1.—	1.50
	leucocytes band forms	»	0.25	0.50	—	1.75
	polymorphs	»	1.—	0.75	—	1.25
	Myeloblasts	»	2.50	3.75	0.25	2.25
	Praemyelocytes	»	4.75	7.50	4.50	1.25
	Neutro- myelocytes	»	26.—	9.75	23.50	5.—
	philes young forms	»	46.25	38.75	42.—	33.25
	band forms	»	4.25	6.25	9.50	14.—
	polymorphs	»	4.25	7.50	7.—	13.50
	Mono- blast	»	—	0.75	—	—
	cytes	»	—	—	—	—
	Lymphocytes	»	3.25	11.75	2.—	21.50
	Plasma & Türk cells	»	0.50	0.50	—	1.—
	Smear cells	»	4.50	7.50	9.—	0.75
	Reticulo endothelial cells	»	0.50	3.50	—	2.75

morphs 35 %, lymphocytes 60 %, monocytes 1 %. Marked anisocytosis, orthochromia, macro-megalo-cytosis. Morphology of the leucocytes normal.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 24—3—46 20 cc. Pernami nr. 169.

*Case 59. I. M. ♂ aged 69. Born 19—10—1877, Tjömö. (Ref. nr. 4675/45).*

*Occupation:* Ex-road-labourer.

In the spring of 1939 progressive lassitude and jaundice. Ewald test meal: ac. 0/2. Hb. 55 %. Made a good recovery under treatment with Pernami. In the summer of 1940 admitted to the 7th Dept. of Ullevaal Hospital for relapse and with his earlier symptoms. Obvious jaundice, tongue smooth, erythrocytes 0.85 mill. rising to 3.9 mill. under treatment with Pernami. Since then perfectly fit till his re-admission to the 7th Dept. in March 1945, having latterly neglected his liver treatment.

Case 59. Cell count and Haemoglobin from ear and sternal blood.

1945	Reticule- cytes per mille	Hmglob. per cent	Erythro- cytes in millions	Nucleated cells		Serum colour
				ear blood	sternal blood	
7—3	5	37	1.65	2600	(9—3) 62600	17
8—3	2					17
10—3	0					
12—3	2	30	1.18	2100		
13—3	17				49200	
14—3	33				22800	15
15—3	70	31	1.19	3200	441500	15
16—3	240				226600	10
17—3	240	35	1.58	5000		
18—3	370				258800	
19—3	230	45	2.12	3400		10
20—3	110					
21—3	70	54	2.82	6200		
23—3	120	53	2.80	6500		6
24—3	40				162800	
26—3	22	56	2.68	4600		
28—3	22	62	2.99	4000		
31—3	20	66	3.24	4000		3
6—4		71	3.60	6400		
30—4	3	93	4.80	4500		
15—5		96	4.80	9300		
26—5		96	4.60	5800		

Case 59. Differential cell count of sternal blood.

1946			9—3	13—3	14—3	15—3	16—3	18—3	24—3
Sternal puncture			Asp. easy 0.7 cc. st. blood m.p. + + + + f.p. + moderate a. pain	Asp. easy 0.5 cc. st. blood m.p. + + + + f.p. + + severe a. pain	Asp. easy 0.3 cc. st. blood m.p. + + + + f.p. + j severe a. pain	Asp. easy 0.25 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. easy 0.1 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. easy 0.3 cc. st. blood m.p. + + + + + f.p. 0 severe a. pain	Asp. easy 0.3 cc. st. blood m.p. + + + + f.p. 0 moderate a. pain
Erythropoiesis	Erythroblasts	per cent	39.2	33.3	46.5	62.5	74.1	52.6	41.6
	Leuco- blasts	"							
	cytes	"	60.8	66.7	53.5	37.5	25.9	47.4	58.4
	Promegalomacroblasts	"	9.5	6.5	3.5	7.5	1.50	2.5	2.5
	Megalobl. bas.	"	6.5	13.5	5.—	—	—	0.5	—
	eos.	"	—	—	—	—	—	—	—
	Macrobl. bas.	"	42.—	53.5	37.5	46.—	13.50	8.—	6.—
	eos.	"	36.—	8.5	53.—	8.—	0.25	1.5	2.5
	Normobl. bas.	"	—	—	—	29.—	66.—	27.5	27.5
	eos.	"	—	—	—	6.5	18.25	59.—	61.—
Leucopoiesis	Erythrobl. bas.	"	1.—	4.—	—	—	—	—	—
	eos.	"	5.—	14.—	—	—	—	—	—
	Erythrobl. bas.	"	—	—	1.—	2.5	0.50	—	0.5
	division forms eos.	"	—	—	—	—	—	1.—	—
	Megacaryocytes	"	—	—	—	0.25	+	—	+
	Mast cells immature	"	—	—	—	0.25	—	—	—
	mature	"	—	—	—	—	—	—	—
	Eos. myelocytes	"	2.50	3.25	2.—	1.25	3.25	1.—	1.—
	leucocytes band forms	"	4.25	2.75	1.50	1.—	2.75	1.—	1.50
	polymorphs	"	1.50	1.—	3.—	1.50	2.—	1.—	0.50
	Myeloblasts	"	3.—	1.—	2.75	1.50	1.75	2.—	1.75
	Pracmyelocytes	"	9.50	5.75	4.50	4.50	8.—	3.25	2.25
	Neutro- myelocytes	"	18.25	10.25	12.75	17.25	15.—	13.25	20.50
	philes young forms	"	33.25	21.25	33.50	49.50	47.25	42.50	39.—
	band forms	"	1.75	4.75	3.—	5.—	2.25	6.50	5.25
	polymorphs	"	1.75	5.—	4.50	5.—	3.75	3.75	7.75
	Mono- blasts	"	}	—	—	—	—	—	0.25
	eytes	"							
	Lymphocytes	"	13.75	25.50	15.—	1.75	2.75	5.25	10.50
	Plasma & Türk cells	"	0.75	—	—	—	—	0.50	0.25
	Smear cells	"	7.50	14.50	15.50	10.75	8.50	17.75	9.—
Reticulo endothelial cells			1.25	5.—	2.—	0.75	2.50	2.25	0.50



Present condition: He is pale yellow, his sclerae jaundiced. Hour-glass nails on all his fingers. Blood press. 130/80. Temp. 37.6. Lymphatic glands, liver and spleen not palpable. Urine: Schlesinger 1/10 +. Pain in legs below the knees. Loss of deep sensation in the lower limbs and absence of Achilles reflexes interpreted as indicating funicular myelosis. A radiological examination showed arteriosclerosis of the legs below the knees, but nothing abnormal in stomach or duodenum. An electrocardiographic examination showed signs of myopathy. Uric acid 10.5 mg. %, platelets 279,000. Cholesterolin 88 mg. %. Takata and formol-gel. ÷. S.R. 44 to 20 mm. Bleeding-time 3 min. Ewald test meal: ac. 0/3. Total proteins 6.1 %, alb. 4.2 %, glob. 1.9 %. Coagulation-time (Gram's coagulometer) 8 to 14 min. Benzdin test of faeces ÷.

*Treatment:* 13—3—45 40 cc. Pernami »Nycos« nr. 177.

25—4—45	20 cc.	»	»	»	»
28—4—45	10 cc.	»	»	»	»
1—5—45	20 cc.	»	»	»	»
5—5—45	20 cc.	»	»	»	»
15—5—45	20 cc.	»	»	»	»

A comparatively intensive treatment with liver injection was given on account of the funicular myelosis. 7—3—45 blood smear from ear: bas. 0 %, eos. 0 %, band forms 4 %, polymorphs 28 %, lymphocytes 62 %, monocytes 6 %. Marked anisocytosis, macro-megalocytosis, poikilocytosis, ovalocytosis, microcytosis, schizocytosis. Orthochromia. Normal morphology of the leucocytes.

*Sternal puncture:* Megalo-macroblastosis.

*Case 60. I. J. ♀ aged 82. Born 17—7—1864, Holmestrand. (Ref. nr. 12224/46).*

*Occupation:* Ex-shopkeeper.

Troubled for some years by increasing dyspnoea, »anginoid« discomfort on exertion. In the spring of 1941 failing appetite, attacks of vomiting, loss of weight, increasing giddiness, tinnitus and soreness of his tongue.

*Case 60. Cell count and Haemoglobin from ear and sternal blood.*

1946	Reticulo-cytes per mille	Hmglob. per cent	Erythro-cytes in millions	Nucleated cells		Aspr. quantity stern. blood
				ear blood	Sternal blood	
18—6	20	45	1.78	7800	>96000	0.4
20—6	36	45	1.64	4500		
24—6	223	48	1.79			
1—7	42	65	2.55	6500		

Admitted May, 1941 to the 8th Dept. of Ullevaal Hospital where pernicious anaemia probably complicated by funicular myelosis or arteriosclerosis was diagnosed. Hb. 47 %, erythrocytes 1.85 mill. Successfully treated with Pernami. In 1944 admitted to the 7th Dept. of Ullevaal Hospital and treated for relapse. Re-admitted 14—6—46. Perfectly conscious, but off and on drowsy. Pain in the knees (arthrosis deformans). Blood press. 175/90. Tongue smooth, clean. Pulse 104, extrasystoles. Lymphatic glands, spleen and liver not palpable. Areflexia of the lower limbs, but otherwise normal findings on an ordinary clinical examination. Electrocardiogram: bigemini, otherwise normal. A radiological examination showed nothing abnormal in stomach or duodenum, but some enlargement of the heart, arteriosclerosis, and infiltration of the right lung. Wassermann ÷. Ewald test meal: Congo ÷. Benizidin test of faeces ÷. Serum colour 4. S.R. 46 mm. Blood smear from ear: eos. 0 %, bas. 0 %, band forms 4 %, polymorphs 62 %, lymphocytes 33 %, monocytes 1 %. Marked anisocytosis, orthochromia, microcytosis, schizocytosis, basophil punctuation. Normal morphology of leucocytes.

*Sternal puncture:* Macro-normoblastic reaction, crisis.

*Treatment:* 19—6—46 to 20—8—46: 35 cc. Pernami.

*Case 61. H. E. ♂ aged 68. Born 22—2—1878, Oslo. (Ref. nr. 8785/46).*

*Occupation:* Ex-mechanic.

Since 1919 attacks of griping abdominal pain independent of meals. In 1931, gastritis polyposa was diagnosed, and the stomach resected at the 3rd Dept. of Ullevaal Hospital. In 1938 admitted to the 8th Dept. of

Case 61. Cell count and Haemoglobin from ear and sternal blood.

1946	Reticulo- cytes per mille	Hmglob. per cent	Erythro- cytes in millions	Nucleated cells	
				ear blood	sternal blood
2—5	2	83	2.25	4400	154000
5—5					
10—5		76	2.48	5000	
3—6		54	2.28	2500	162400
5—6	4				
11—6	56	60	2.32	5700	
12—6	58				218000
13—6	70				
14—6	42				
18—6	15	69	2.65	4100	
20—6	4				
2—7		79	2.98	10000	

Case 61. Differential cell count of sternal blood.

1946			5—5	5—6	20—6
Sternal puncture			Asp. easy 0.2 cc. st. blood m.p. + + + + + f.p. + + + + +	Asp. easy 0.5 cc. st. blood m.p. + + + + + f.p. + + + + +	Asp. easy 0.4 cc. st. blood m.p. + + + + + f.p. + + + + + moderate a. pain
Erythropoiesis	Erythroblasts	per cent	38.70	39.90	24.10
	Leuco-                      blasts	»			
	cytes	»	61.30	60.10	75.90
	Promegalomacroblasts	»	18.50	16.50	2.—
	Megalobl.                      bas.	»	5.50	6.50	—
	eos.	»	—	—	—
	Macrobl.                      bas.	»	58.50	57.—	16.50
	eos.	»	7.50	8.50	0.50
	Normobl.                      bas.	»	—	1.—	74.50
	eos.	»	—	—	5.—
	Erythrobl.                      bas.	»	3.50	1.—	—
	eos.	»	5.—	6.50	—
	Erythrobl.                      bas.	»	1.50	3.—	1.50
	division forms                      eos.	»	—	—	—
Leucopoiesis	Megacaryocytes	»	—	0.50	—
	Mast cells                      immature	»	—	0.25	—
	mature	»	—	—	—
	Eos.                      myelocytes	»	0.75	0.75	0.50
	leucocytes                      band forms	»	1.25	1.75	0.75
	polymorphs	»	2.—	2.50	—
	Myeloblasts	»	4.25	1.25	1.50
	Praemyelocytes	»	5.25	5.—	11.25
	Neutro-                      myelocytes	»	12.75	18.75	20.75
	philes                      young forms	»	40.25	36.50	42.—
	band forms	»	9.25	5.50	9.75
	polymorphs	»	10.—	9.—	5.25
	Mono-                      blasts	»	—	—	—
	cytes	»			
	Lymphocytes	»	9.75	13.75	4.25
	Plasma & Türk cells	»	1.—	1.—	—
	Smears cells	»	2.50	2.75	2.50
	Reticulo endothelial cells	»	1.—	0.75	1.50

Ullevaal Hospital on account of lassitude. Pernicious anaemia diagnosed. Hb. 53 %. Liver treatment successful. Re-admitted to hospital in 1944 because liver treatment had been inadequate. Treatment with Pernami successful. Admitted to the 7th Dept. of Ullevaal Hospital 26—4—1946.

*Present condition:* Mentally deranged and partially unconscious, reliable data could not be obtained from him. Blood press. 200/105. Patellar and Achilles reflexes could not be evoked, and the plantar reflexes inverted. Some dysarthria. His pernicious anaemia seems to be complicated by a funicular myelosis and senile dementia. Radiological examination of the colon, stomach and duodenum negative, sclerosis of the aorta. An electrocardiographic examination shows myopathy. The benzidin test of the faeces + after a few seconds. Wassermann ÷. Total proteins 6.5 %, alb. 4.8 %, glob. 1.7 % (11—5—1946). Serum colour 5. Erythrocytes osmotic resistance: commencing haemolysis at 0.48 % NaCl., total haemolysis at 0.32 %. Urine: Schlesinger (1/10) + S.R. 22 mm. Blood smear from ear: eos. 1 %, bas. 1 % band forms 3 %, polymorphs 66 %, lymphocytes 25 %, monocytes 4 %. Anisocytosis, macrocytosis, orthochromia, micro-schizocytosis. Normal morphology of the leucocytes.

*Treatment:* 5—6—46 40 cc. Pernami »Nyco« nr. 187.

*Case 62 D. B. ♂ aged 77. Born 20—5—1869, Sweden, (Ref. nr. 7510/45).*

*Occupation:* Ex-clerk.

Pernicious anaemia diagnosed in 1938, and since then liver injections at

Case 62. Cell count and Haemoglobin from ear and sternal blood.

1945	Reticulo-cytes per mille	Hmglob. per cent	Erythro-cytes in millions	Nucleated cells		Serum colour
				ear blood	sternal blood	
20—1	3	62	2.56	.6200	.41800	9
25—1		60	2.38	7900		
28—4						8
15—5		71	2.75	3500		
31—5	20	79	3.20	6400		5
9—6	2	83	3.75	7900		
21—6		86	3.50	6800		
25—6	6	87	3.60	5600		
28—6	14	87	3.62		5	
1—7	36					
2—7	80					
3—7	48	96	4.30	5600		
6—7	18	97	4.20	6000		
12—7	12	99	4.10			

Case 62. Differential cell count of sternal blood.

1946			25-6	12-7
Sternal puncture			Asp. easy 0.1 cc. st. blood m.p. +++ f.p. +++ moderate a. pain	Asp. easy 0.5 cc. st. blood m.p. +++ f.p. +++ moderate a. pain
Erythropoiesis	Erythroblasts	per cent	23.2	18.1
	Leuco-      blasts	"		
	cytes	"	76.8	81.9
	Promegalomacroblasts	"	1.5	5.—
	Megalobl.      bas.	"	—	—
	eos.	"	—	—
	Macrobl.      bas.	"	28.—	7.—
	eos.	"	22.5	1.—
	Normobl.      bas.	"	3.—	20.—
	eos.	"	26.5	66.—
	Erythrobl.      bas.	"	7.5	—
	eos.	"	8.5	—
	Erythrobl.      bas.	"	1.5	—
	division forms eos.	"	1.—	1.—
	Megacaryocytes	"	—	0.25
	Mast cells      immature	"	—	0.25
	mature	"	—	0.25
Leucopoiesis	Eos.      myelocytes	"	1.50	0.75
	leucocytes      band forms	"	1.—	0.50
	polymorphs	"	0.25	0.75
	Myeloblasts	"	1.25	1.25
	Praemyelocytes	"	2.25	5.75
	Neutro-      myelocytes	"	14.75	14.25
	philes      young forms	"	32.25	33.25
	band forms	"	6.75	7.25
	polymorphs	"	16.75	13.25
	Mono-      blasts	"	—	1.—
	cytes	"	—	—
	Lymphocytes	"	16.50	16.25
	Plasma & Türk cells	"	—	0.50
	Smear cells	"	5.50	4.50
	Reticulo endothelial cells	"	0.75	—

monthly intervals. Of late progressive lassitude and pain in the chest radiating to both arms, associated to some extent with giddiness. Of late failing appetite and loss of weight. Sleeps badly. Signs of hypertrophy of the prostate. No liver injections the last three years. Admitted to the 7th Dept. of Ullevaal Hospital 18—4—1945.

*Present condition:* Thin and pale. Temp. 37.7. Blood press. 190/100. Tongue moist, clean, not smooth. Palpable arteriosclerosis. Liver, spleen and lymphatic glands not palpable. Presystolic and diastolic murmurs, division of second heart sound along the left border of the sternum. Normal conditions found on a neurological examination. A radiological examination showed enlargement of the heart, calcified deposits in the valves of the aorta, sclerosis of the aorta. An electrocardiographic examination showed myopathy, bundle-branch block. Serum colour 9. S.R. 40 to 15 mm. Non-protein nitrogen 50 mg. %. Cholesterol 136 mg. %. Takata and formal-gel. normal. Total protein (serum) 6.8 %, alb. 4.2 %, glob. 2.6 %. Uric acid 2.1 mg. %. Urine: Schlesinger (1/10) ÷. Wassermann ÷. Ewald test meal: ac. 0/2. Benzidine test of faeces ÷. Blood smear from ear: bas. 0 %, eos. 1 %, band forms 2 %, polymorphs 67 %, lymphocytes 26 %, monocytes 4 %.

*Sternal puncture:* Megalo-macroblastosis.

*Treatment:* 25—6—45 40 cc. Pernami «Nyco» nr. 179.

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# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CCXIX (219)

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## Health hazards in the chloroprene rubber industry and their prevention

*A clinical and experimental study with special  
reference to chloroprene as well as oxidation  
and polymerization products thereof*

By

ÅKE E. NYSTRÖM

*Med. lic., fil., kand.*

ACCOMPANIES VOL. CXXXII (132)

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STOCKHOLM 1948

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From the Industrial Hygiene Department of the Swedish Public Health Institute  
Head: Professor Sven Forssman, M. D.

# HEALTH HAZARDS IN THE CHLOROPRENE RUBBER INDUSTRY AND THEIR PREVENTION

A clinical and experimental study with special reference  
to chloroprene as well as oxidation and  
polymerization products thereof

BY

ÅKE E. NYSTRÖM

*Med. lic., fil. kand.*

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STOCKHOLM 1948



*To my parents*



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## Preface.

The work reported here has been carried out at the Industrial Hygiene Department in the Swedish Public Health Institute. I desire to convey here my great gratitude to the head of that department, Professor Sven Forssman, M.D., who was the first to arouse my interest in the problems of occupational hygiene in the synthetic rubber industry, and who has given me valuable advice and directions during the whole course of the work.

For fruitful discussions and suggestions, I wish to convey my thanks to the laborator at the above-mentioned department, Docent Axel Ahlmark, M.D., the laborator at the University of Uppsala, Dr. Henrik Enghoff, M.D., and to Docent Torgny Sjöstrand, M.D., at Karolinska Institutet.

For valuable help in the microscopical examination of animal organs, I am indebted to the prosector at Karolinska Institutet, Docent Gösta Hultquist, M.D.

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It is a pleasure for me to convey my thanks to Dr. phil. Andreas Klit of the Swedish Rubber Research Laboratory at Uppsala and the chemists and engineers attached to the Department for Industrial Hygiene for valuable discussions regarding the chemistry of chloroprene.

On repeated occasions during the years in which this investigation has proceeded, my work has been carried on at the synthetic rubber factories in Sweden. I beg to thank the managers, engineers and other staff for the interest and complaisance they have always shown me. In connection with the medical examinations at the factories I beg to thank Dr. Johan Wersäll, the county medical officer, for valuable discussions.

I owe special thanks to my assistant at the Industrial Hygiene Department, Mr. Rune Cederlöf, for his interested and careful work.

As regards the translation, I wish to thank Mr. Grenville Grove for all the interest he has shown and the care he has taken.

This investigation has been facilitated by financial support from the Swedish Employers' Confederation and the Swedish Trades Union Federation, for which I desire to express my gratitude.

Stockholm, November 1948.

*Åke E. Nyström.*

## Introduction.

The production of synthetic rubber on an industrial scale is of comparatively recent date. In Sweden this production was not taken up until the last world war, when the reduction of imports entailed a serious shortage of raw rubber. Among the various methods available for the production of synthetic rubber, the one based on the synthesis of chloroprene was considered to be most suitable for this country. The industrial application of this synthesis involved, in many respects, new and difficult problems. Moreover, in many cases experiences from similar industries in foreign countries were not available, as they were usually not published in view of the military importance of the rubber industry. This applied also to the problems of occupational hygiene in connection with this production. Certain questions of this nature had indeed been elucidated by the work of v. OETTINGEN, HUEPER, DEICHMANN-GRUEBLER and WILLEY (1936) on the toxicology of chloroprene, but, being an isolated investigation, based on animal experiments, it did not deal with several problems relating to industrial medicine and to occupational hygiene, that pressed for solution soon after this industry had started. In fact, it became manifest at an early date that the health of the employees were exposed to injurious effects by the work in these factories. In view of the great importance of the rubber industry for this country under the existing conditions, it had become very urgent to try and ascertain the causes of the ill-effects on the health of the workers and, if possible, to eliminate them, so that an undisturbed production could be maintained.

The problems that seemed most important to the author, who had been entrusted with this task, and which determined the planning of this work, were the following: —

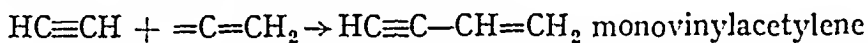
1. Are there any indications that the work in the synthetic rubber industry injuriously affects the health of the employees?

2. What substance or substances or stages in the process of production can be considered to cause deterioration in health?
3. Do experiments on animals with the substance or substances in question show that they are injurious to the animal organism?
4. Is there any correlation between the observations from such experiments and the symptoms found among the workers?
5. What measures can be taken to reduce the risks of ill-health in this industry?

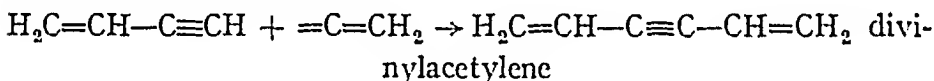
## CHAPTER I.

### CHLOROPRENE AND ITS POLYMERS.

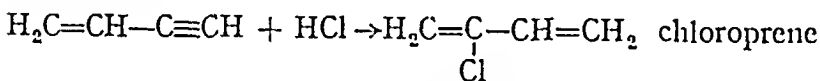
It is known from investigations made at the beginning of the present century that, if acetylene is passed through saturated solutions of cuprouschloride in sodium- or ammoniumchloride, monovinylacetylene and divinylacetylene are formed. This reaction is due to the polymerization of acetylene under the action of cuprouschloride as a catalyzer (NIEUWLAND, CALCOTT, DOWNING, CARTER 1931, NIEUWLAND and VOGT 1945). It is considered that activated acetylene, probably  $=C=CH_2$ , reacts with normal acetylene, forming monovinylacetylene:



This reaction proceeds under the action of acetylene, whereupon divinylacetylene is formed:



By the addition of hydrogenchloride to the vinylacetylene under favourable conditions, the product formed consists almost solely of chloroprene:



Chloroprene is a colourless liquid with a characteristic smell, somewhat resembling that of ethylbromide. Its specific weight at  $20^\circ \text{C}$  is 0.9583, and at a pressure of 760 mm. Hg it boils at  $59.4^\circ \text{C}$ . The flash-point of chloroprene is  $-10^\circ \text{C}$ , its molecular weight 88.46. It is soluble in ordinary organic solvents such as benzol, alcohol, ether, chloroform, etc. (As to its solubility in water, see Ch. IV.)

According to investigations by KLIT (unpublished) and others,

chloroprene after a certain induction period absorbs oxygen at a rate of about 1 volume of oxygen per volume chloroprene and per minute. After addition of pyrocatechin the absorption of oxygen is reduced to ca. 2—3 per cent of that value. Detailed particulars as to which products are formed in the absorption of oxygen could not be found in the literature. It has, however, been shown that acid is produced, probably, however, bound like a kind of lactone. Chloroprene has a marked tendency to polymerization, a process that takes place spontaneously and rapidly even at room temperature.

If chloroprene is allowed to stand at ordinary room temperature with the access of air, a polymer termed  *$\alpha$ -polychloroprene* is first formed. It can be isolated by precipitation with alcohol or by the distillation in a vacuum. It is plastic, that is to say it easily changes its form on compressive or tensile strain and then retains this change of form. It is soluble in benzol. At a temperature of about 30° C and with the access of air the  *$\alpha$ -polymer* loses its plastic properties and in the course of twenty-four hours is almost completely changed into another polymeric form, the  *$\mu$ -polychloroprene*.

The  *$\mu$ -polymer* is colourless or pale yellow, transparent and elastic, resembling soft vulcanized rubber. It is sparingly soluble and swells only under the action of carbontetrachloride, carbon-disulphide, benzol, ether and a few other solvents. Under the action of air the  *$\mu$ -polymer* gradually gets darker in colour and after two or three weeks it is dark brown. At the same time it gets harder. This change is attributable to autoxidation and can be inhibited by treatment with anti-oxidation substances.

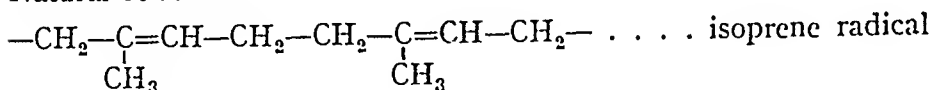
If chloroprene is polymerized at higher temperature (about 60° C) and in the absence of oxygen,  *$\beta$ -polychloroprene* is formed. In the distillation of this product two fractions are obtained. The one distils at 92—97° C at a pressure of 27 mm. Hg and the other at 114—118° C at the same pressure. Both have the characteristic terpenelike smell of the  *$\beta$ -polychloroprene*. The  *$\beta$ -polymer* is a relatively stable product, which has no tendency to polymerize further. For this reason, it is of no great importance in the rubber industry.

Under certain conditions the exact nature of which is not known, chloroprene yields a polymer, termed  *$\omega$ -polymer*, of granular structure. It consists of a rather hard mass of small lustrous rubber

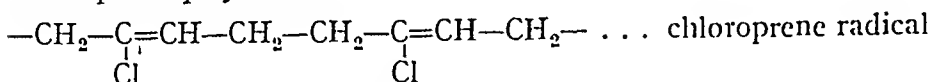
granulae. Chloroprene can also yield several other polymers if the conditions for the polymerization process are modified. The exact nature of these polymers, however, is not known.

In analogy with the chemical structure of natural rubber, the molecules of which are built up of long chains of isoprene radicals, it is considered that the chloroprene polymers consist of chloroprene radicals linked into chains, viz:

Natural rubber:



Chloroprene polymers:



Whereas the chain-formation of isoprene radicals is a rather slow process, the corresponding reaction of the chloroprene radicals proceeds much more rapidly; this is partly attributable to the Cl-atom, which serves as an activator.

The  $\alpha$ -polymer is considered to have a purely linear structure with the chloroprene radicals arranged in long chains, whereas the  $\mu$ -polymer is supposed to have a tridimensional structure with the chains arranged in the form of "rings". This structure of the  $\alpha$ - and  $\mu$ -polymers corresponds well with the different reaction of these substances to other chemical substances. The  $\mu$ -polymer is much more resistant to chemical action than the  $\alpha$ -polymer. The exact chemical constitution of the  $\beta$ -polymer has not yet been ascertained, but there are many indications that it consists of a cyclic dimer of chloroprene. We have an analogous product of isoprene with such a structure. In view of the small reactive tendency in the  $\omega$ -polymer, it may be presumed that the "ring"-formation of the chains in this polymer has been carried rather far.

It is pointed out in the literature that the above-described polymers should not be regarded as distinct chemical individuals, but as mixtures of polymeric modifications where the one or the other modification predominates and determines the characteristic features of the polychloroprene (for the literature, see CAROTIERS, WALLACE, WILLIAMS, COLLINS, KIRBY 1931, CAROTIERS, COFFMAN 1932 and ELLIS 1935).

In connection with the above description of the synthesis of



chloroprene, a brief survey will now be given of the production of synthetic rubber on a manufacturing scale according to this process.

Acetylene is produced in the usual way from carbide and water at the acetylene generator (A) and is then transferred to a contactor (B), where monovinylacetylene and, in a smaller amount, divinylacetylene are formed under the action of a catalyst. These

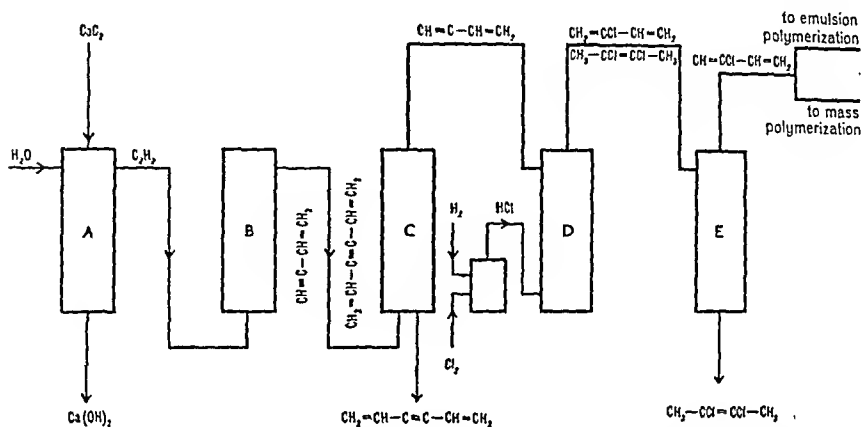


Fig. 1. — Sketch of the process for the manufacture of synthetic rubber.

two substances are then transferred to a column (C), where the divinylacetylene is separated. The monovinylacetylene is then transmitted to a second column (D), where, under the action of hydrogenchloride, it is partly re-formed into chloroprene and partly, in a smaller amount, into dichlorobutene, from which the chloroprene in the following column (E) is separated by fractional distillation. It is then forwarded to the polymerization departments. For the polymerization of chloroprene different procedures may be employed: the principal methods adopted in Sweden are emulsion polymerization and mass polymerization. Mass polymerization is carried out on a slowly rotating stainless steel conveyor, on which chloroprene is spread out in a thin film.

## CHAPTER II.

### THE MEDICAL ASPECT OF OCCUPATIONAL PROBLEMS IN CONNECTION WITH THE PRODUCTION OF SYNTHETIC RUBBER IN SWEDEN.

As previously mentioned, it was found rather soon after the production of synthetic rubber on a manufacturing scale had started that the workers were exposed to injurious effects from their work. At the beginning of 1944 the present author made the first investigations on this subject at a small pilot plant, which had then been running for barely a year. Even at this plant it seemed as though the risks in this respect were greater in certain departments than in others. But, in view of the fact that the same workers, from time to time, might be employed at several different places in the plant, it was difficult to locate the precise source of the trouble. In subsequently started factories, where the operation was more rationally organized, and where the workers, generally speaking, were obliged to confine themselves to one stage in the work, it became evident, however, that only the workers in certain departments had symptoms of ill-health.

Among the symptoms that manifested themselves among the workers in the rubber industry, an adverse change in the general condition was most common and most marked. It was especially the workers in the department for fractional distillation that complained about this. It was found that 90 per cent of the workers (21) in this department considered that they had got much more fatigued there than by their previous employment in other branches, though they themselves classed the work in the synthetic rubber industry as light manual labour. The work in fact largely consists in the handling and control of apparatuses of different kinds. The

fatigue set in about one month after starting work in the said department, but in some cases after a shorter lapse of time. This feeling of fatigue made itself particularly felt after the end of the day's work but often persisted till the following morning, though it was then much less marked. Among the workers there were active sportsmen, whose athletic performances fell off considerably, so that as a rule they were obliged to give up their athletics after employment for some time in this industry.

In a great many cases the fatigue was combined with a sensation of oppression diffusely spread over the chest. It was noticeable even at a steady pace of work, but became more marked in case of intensified effort. Many of the workers then complained of pain, which was sometimes so intense that it was described as "severe" and was localized under the sternum from the epigastrium up to the region of the larynx. This symptom was strictly localized in the said region, and no radiation to shoulder parts, back or arms has been reported. Like the fatigue, these symptoms became most marked towards the end of the day's work, and the workers often had great difficulty in going from the factory to their homes. In many cases they had to rest repeatedly on their way home (often by cycle), and afterwards they were as a rule incapable of doing any more physical work on that day. The symptoms usually subsided before the next day's work was to begin, but some of the men had difficulties in cycling even to their work. After that the work-men had been away from their work for a few days, the symptoms for the most part vanished, but in case of intensified effort they might recur a week, a fortnight or more after the cessation of the factory work. The feeling of oppression over the chest recurred among about 90 per cent of the workers in the fractional distillation department and about 10 per cent of the workers (12) in the polymerization department.

About 25 per cent of the workers in the fractional distillation department were troubled with palpitation even after a slight exertion. This, however, was not a constant symptom, but usually manifested itself after these workers had been exposed to the effects of chloroprene for a considerable length of time.

About 30 per cent of the men in the distillation department believed that, owing to the work there, they had undergone a marked change of disposition. They had become irritable, peevish

and quick-tempered. A similar view was held by those around them. As these men are included in the above-mentioned group who believed that their general condition had changed for the worse, the greater irritability may possibly be a manifestation of general fatigue. The workmen themselves, however, did not consider this to be the real cause, but attributed the irritability to the effect of gases of some kind.

Approximately 30 per cent of the men in the distillation and polymerization departments, when medically examined, showed signs of dermatitis. As a rule, the disease was very mild and did not cause much discomfort. For the most part it was located in places on the body which had been brought into direct contact with fluid chloroprene, such as the hands, as also the legs after the clothes had become moist with chloroprene. The author in no case observed any spread of the eruption from these spots, and as a rule it receded rather rapidly if the men protected themselves from direct contact with the chloroprene.

The most striking of the symptoms among the workers in the synthetic rubber industry was the loss of hair. This occurred in about 90 per cent of the workers in the mass polymerization department. As a rule it began at the earliest about one month after the beginning of the employment, but proceeded very rapidly once it had started. Rather often it led to complete baldness. Loss of hair on other parts of the body has not been observed. Even those men who had tried to protect themselves from direct contact with the chloroprene by wearing headgear had nevertheless lost their hair to the same extent. If the workers in this department were transferred to another section or were temporarily freed from work in the factory, the hair began to grow again after about two months. If they tried to resume their former work, the hair again began to fall off. There are workers who have lost their hair in this way up to seven times.

The above-mentioned symptoms developed after continuous exposure to chloroprene for a considerable length of time. Acute cases of poisoning have also occurred in this industry, one of them with a fatal issue (see Ch. XI). These cases, however, should be regarded as accidents due to special circumstances, in which the workers had been exposed for a short time to a particularly intense

effect of chloroprene. As a rule the men had then fallen into a state of unconsciousness, which, however, soon passed off. Generally speaking, the recovery of consciousness was not attended by any special discomfort, and the men were able to resume their work immediately.

## CHAPTER III.

### EARLIER INVESTIGATIONS.

Hitherto merely three<sup>1</sup> works on the toxicology of chloroprene have been published, only one of which, by v. OETTINGEN et al., deals thoroughly with the problem. The other two works, by SCHWARTZ (1945) and RITTER and CARTER (1948), are confined to a report on cases of dermatitis and loss of hair among workers employed on the production of chloroprene rubber.

The first-mentioned work is based on animal experiments and is an investigation into the toxic effects of chloroprene at different concentrations and with different methods of administration, as well as a study of the histological organic changes in animals exposed to chloroprene. In the latter part of that work, the authors try to give an explanation of the mechanism of the injurious effects of chloroprene.

As experimental animals the authors used mice, rats, cats and pigeons; they administered the chloroprene cutaneously, subcutaneously, orally and by inhalation. The investigations have made it clear that chloroprene is reabsorbed and has a strong toxic effect, whichever of these methods of administration is adopted.

In the *cutaneous application*, chloroprene was rubbed into the dorsal skin of rats daily for nearly two months. These rats rather soon showed a marked decrease in weight, and at the post-mortem moderately pronounced degenerative changes were found in the liver. The kidneys showed slight signs of nephrosis and the spleen as a rule was hyperemic. It was observed that the hair at the spots which had been exposed to direct contact with the chloroprene was very brittle.

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<sup>1</sup> Since the termination of this investigation, a work by ROUBAL (1942) on the toxicology of chloroprene has become known to the present author. (See the observations on that work towards the end of this chapter.)

In discussing the toxicity of chloroprene after *subcutaneous injection*, the authors point out the difficulty of injecting small amounts, as they do not know any solvent for chloroprene that does not itself entail marked physiological effects. With this administration in rats, the authors found that the "minimum fatal dose" (M.F.D.)<sup>1</sup> was 0.02 ml per g body-weight. It was found that, with the same amount of chloroprene, the animals died after very different times reckoned from the administration, which the authors attributed to a slow and irregular absorption from the place of injection. In subcutaneous injection into pigeons, the authors found a M.F.D. of the same magnitude as for rats, whereas in corresponding experiments on cats this dose was about a hundred times less.

All the animals were strongly affected by the chloroprene. Their respiration rate slowed down and became irregular. After a time a rather marked dyspnea developed together with cyanosis, and some of the animals had asphyxial convulsions, from which they died. The postmortem findings showed, in general, a rather marked hyperemia in the organs, with more or less extensive hemorrhagic areas. In the liver hemorrhagic necroses were observed in a number of cases, and in less acute cases the liver cells formed homogeneous hyaline masses. The kidneys were also affected and showed degenerative changes of the tubular epithelium as well as small hemorrhages in the glomeruli.

In *oral administration* on rats, the authors found the M.F.D. to be 0.4 ml. per rat. As in subcutaneous injection, the times that elapsed between the administration of chloroprene and the death of the animals showed great variations. At postmortem, signs of a marked inflammation in the stomachal cavity and guts were observed, indicated by a considerable swelling of the mucosa, with hemorrhagic areas and ulcerations. The other organs, broadly speaking, showed similar changes to those reported by the authors in connection with the subcutaneous injection.

In determining the M.F.D. for mice on *inhalation* of chloroprene for 1 hour, the value 3 mg per litre of air was obtained, but on exposure for 8 hours the corresponding figure was 0.6 mg. The determination of the M.F.D. for rats showed greatly varying

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<sup>1</sup> MFD "is taken as the amount necessary to cause between 70 and 100 per cent of the animals to die acute deaths" (v. OETTINGEN).

values. The authors, however, consider it ascertained that the M.F.D. for rats lies between 15 and 21 mg per litre of air.

In the composition of the blood, v. OETTINGEN et al. found merely slight changes after the animals had been exposed to chloroprene. Any certain changes in the hemoglobin value or the number of erythrocytes could not be discovered, nor did there occur any effect on the coagulation of the blood.

The effect of chloroprene on the blood pressure and respiration was studied by the authors in experiments on rabbits and cats. After less irregular variation in blood pressure at the beginning of the tests, a continuous fall set in, which the authors attributed to a dilatation of the vessels in the abdomen. The respiration, relatively speaking, was quite slightly affected. A moderate decrease of the respiration rate occurred, however, towards the end of the tests, but even when the blood pressure had fallen to low values, the respiration was almost normal.

v. OETTINGEN et al. do not precisely state the quality<sup>1</sup> of chloroprene used in their experiments on animals. They do not seem to have taken into account the rather considerable change in pharmacological effect that occurs on the oxidation or polymerization of chloroprene (see Ch. V, VII and X). As, in particular, it is very difficult to avoid oxidation in working with chloroprene, it seems by no means improbable that the investigations of the said authors do not refer to identically the same quality of chloroprene. On this assumption, certain differences in the results obtained, e. g. in the mortality determinations, would be quite explicable.

On subcutaneous injection of chloroprene into rats v. OETTINGEN found the M.F.D. to be 0.02 ml per g of the body-weight, and on oral administration 0.4 ml per rat. As in the case of oral administration only the total dosage per rat has been reported without information about the weights of the animals, it is difficult to make a comparison; at all events, however, the oral dosage seems to be remarkably small as compared with the subcutaneous, whence it is difficult to believe that they are concerned with pharmacologically equivalent chloroprene. Moreover, as the experiments were

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<sup>1</sup> By the term "different chloroprene qualities" in this work, the author present refers to chloroprene which, owing to oxidation or polymerization, had changed with respect to its chemical, pharmacological or physiological properties.



as a rule made on series with a small number of animals, the stated results seem in several cases to be doubtful.

SCHWARTZ reports the occurrence of dermatitis among the workers in the chloroprene industry, as well as cases of loss of hair. The present author, in a preliminary communication (1946) has pointed out that falling-off of the hair occurs only when the workers come into contact with the polymers of chloroprene. The same view is taken by RITTER and CARTER (1948), who also consider themselves to have shown that only certain cyclic polymers with short chains are responsible for the loss of hair.

ROUBAL (1942), in a work that came to the knowledge of the present author after the termination of this investigation, reports his researches on the toxicology and pharmacology of chloroprene and describes certain symptoms of disease among the workers employed in the synthetic rubber industry in Czechoslovakia. As regards experiments on animals, ROUBAL found, broadly speaking, the same effects of chloroprene as had previously been noted by v. OETTINGEN et al. in similar experiments. He also examined the effect of polymerized chloroprene on the respiration and heart action of rabbits after injection, but in that regard failed to observe anything noteworthy.

In the medical examination of the workers in the chloroprene industry, ROUBAL several times observed ocular symptoms in the form of conjunctivitis and, in two cases, injury to the corneal epithelium. No symptoms of a similar nature have been noted in the present author's material. This may be due to the fact that the technical procedure in the production of synthetic rubber in certain respects was different (personal communication from ROUBAL). Thus, ROUBAL attributes the corneal injuries to the effects of methylvinylketone ( $\text{CH}_3\text{—CO—CH=CH}_2$ ), a compound to which the workers had not been exposed to any appreciable extent in the manufacturing process in Sweden.

As in Sweden, falling-off of the hair occurred also among the workers in the Czechoslovak rubber industry. It is noteworthy that ROUBAL had observed a loss of hair also from the eyebrows as well as a slower growth of the beard, a symptom which the present author has not noticed among the workmen in the Swedish rubber industry. This may possibly be due to differences in the degree and duration of the exposure. To what substances





# OWN INVESTIGATIONS

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# Studies on the Nature of the Chloroprene.

## CHAPTER IV.

### CERTAIN PROBLEMS OWING TO THE CHEMICAL AND PHYSICAL PROPERTIES OF THE CHLOROPRENE.

Toxicological tests of chloroprene present certain special problems owing to the chemical and physical properties of that substance. As pointed out by v. OETTINGEN et al., it is difficult to ascertain the effect of small amounts of chloroprene, on account of its slight solubility in pharmacologically inactive solvents. The present author has been unable to find any exact data in the literature in regard to the *solubility of chloroprene in water*. It is indeed stated to be sparingly soluble, but no figures are given. In determining its solubility in water by computing the index of refraction, the author set out from newly distilled chloroprene, stabilized with pyrocatechin. Before the determination, the chloroprene was shaken with distilled water for 15—20 hours. Under these conditions, the solubility was found to be about 0.05 ml chloroprene per 100 ml water at 20° C.

A question with which one is often confronted in experimental tests of chloroprene on animals, is the state *degree of polymerization* of the chloroprene on a certain occasion. In default of any satisfactory, practicable method for determining the degree of polymerization, it is often difficult to answer this question. Certain facilities for following the course of the polymerization are, however, afforded by viscosity determinations, even if there is not always a simple relation between the viscosity and the degree of polymerization. The author found that the viscosity of chloroprene changed

uniformly during the first few hours after distillation if the chloroprene was kept at room temperature ( $18^{\circ}\text{C}$ ) and without being sheltered from light or air. But after about 6 hours constant increasing values for the viscosity could as a rule no longer be obtained. As pointed out in describing the polymers of chloroprene, it is almost solely  $\alpha$ -polychloroprene that is first formed at ordinary room temperature. Not until its concentration has reached about 25 per cent is the  $\mu$ -polymer formed. Even if the supposition that the viscosity may be proportional to the length of linearly linked molecules has not been left unchallenged (for the literature see ELLIS, 1935), the present author nevertheless considers it justifiable to regard the above-described uniform change in the viscosity as an indication of a continuous formation of the  $\alpha$ -polymer. On the formation of the  $\mu$ -polymer the uniform change in the viscosity is apparently interrupted, which is in conformity with the fact that the viscosity is irregularly changing in the presence of molecules of ring structures (STAUDINGER and OCHIAI, 1931).

In the viscosity determinations, the author used a Höppler viscosimeter. In such determinations one records the falling-time of an excentrically falling ball in an obliquely placed glass tube containing the fluid to be examined. The records were taken at  $20^{\circ}\text{C}$ .

The falling-times in tests with chloroprene immediately after distillation and during the next few hours are recorded in the subjoined table. Chloroprene (designated I, II and III), after distillation, was kept at room temperature ( $18^{\circ}\text{C}$ ) with access of air and without being sheltered from light. For purpose of comparison, the same table gives analogous figures for chloroprene (designated IA, IIA and IIIA) kept after distillation under other conditions: higher temperature ( $25^{\circ}\text{C}$ ) and sheltered from light and direct access of air. The determinations refer to chloroprene distilled on 6 different occasions.

These determinations indicate that the falling-times fluctuate rather considerably even after a comparatively small change of the temperature and if the conditions in regard to light and access of air are at the same time modified, but that they vary with great regularity up to a certain time as regards chloroprene kept under identical conditions.

In view of the great affinity of chloroprene for oxygen and its marked tendency to polymerization, it was considered desirable

Table I. The falling-times in tests with the chloroprene immediately after distillation and during the next few hours. The falling-times are expressed in minutes and seconds.

Time in hours after distillation	I	II	III	IA	IIA	IIIA
0	0' 47"	0' 46"	0' 47"	0' 46"	0' 48"	0' 47"
1	0' 48"	0' 49"	0' 49"	0' 56"	0' 59"	0' 56"
2	0' 51"	0' 52"	0' 52"	1' 34"	1' 31"	1' 32"
3	1' 02"	1' 04"	1' 02"	1' 51"	1' 50"	1' 47"
4	1' 19"	1' 19"	1' 23"	2' 01"	2' 09"	2' 11"
5	1' 26"	1' 27"	1' 30"	2' 07"	2' 09"	2' 16"
6	1' 30"	1' 45"	1' 58"	2' 10"	2' 20"	2' 46"

to ascertain whether a progressive oxidation or polymerization affected the *rate of evaporation*.

In fact, in animal experiments proceeding for several hours, one must reckon with oxidation and also with some polymerization. The latter, however, can be obviated by a suitable stabilizer. The author studied the evaporation rate (1) of chloroprene stabilized with pyrocatechin and (2) of non-stabilized chloroprene. In these determinations the same apparatus as in the inhalation tests was employed (see Ch. V B). The determinations were made at room temperature (18°—19° C) and the velocity of the air flowing through was maintained at 1.15 litres per minute. During the first hour after the distillation the amount of evaporated chloroprene was computed every 10th minute, and afterwards every hour up to 8 hours. It was found that the stabilized chloroprene evaporated at the same rate during the whole observation time. In tests with stabilized chloroprene that had been kept for one week, the author noted a decrease in the amount evaporated by about 75 mg per hour. This change in the rate of evaporation should presumably be attributed to the development of  $\beta$ -polymers, the formation of which is not prevented by pyrocatechin. In non-stabilized chloroprene the evaporation during the first hour after distillation was, broadly speaking, directly proportional to the time, but afterwards diminished according as the viscosity increased. Thus, during the 6th hour after distillation the amount evaporating was merely about one-fourth of that during the first hour.

These tests show that the oxidation of chloroprene does not seem to change the rate of evaporation and that constant concentrations

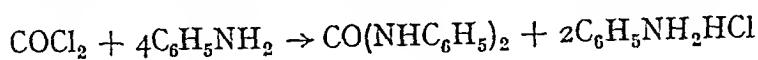


of pyrocatechin-stabilized chloroprene can still be obtained 8 hours after distillation. With non-stabilized chloroprene, on the other hand, this is the case only during the first hour, after which the rate of evaporation rapidly diminishes.

It is generally known that several saturated as well as non-saturated hydrocarbons under certain conditions are decomposed, in which process *the formation of phosgene*, amongst other products, occurs (JACOBS, 1944, and others). This occurs e. g. when a flame is burning in air containing fumes of chlorinated hydrocarbons or when those compounds are exposed to ultraviolet light. TANDBERG (1939), among others, has shown that the amount of phosgene formed in the above-mentioned way may be considerable and attain dangerous concentrations. In tests with an open flame, TANDBERG obtained 29 mg of phosgene and in ultraviolet radiation 220 mg per g trichlorethylene; and in ultraviolet radiation of 1 g perchlorethylene up to 297 mg of phosgene. Presumably such favourable conditions for the formation of phosgene from chlorinated hydrocarbons occur very rarely under ordinary conditions. But even under less optimal conditions phosgene may be produced from chlorinated hydrocarbons; moreover, carbon-tetrachloride, trichlorethylene and chloroform, kept under ordinary laboratory conditions, rather often contain minor amounts of phosgene. Furthermore, a number of cases of poisoning which must indubitably be attributed to the effect of phosgene produced in the decomposition of chlorinated hydrocarbons are reported in the literature (JOHNSTONE, 1948, and others).

The author has been unable to find any report that the formation of phosgene had been observed in the decomposition of chloroprene. From a theoretical point of view, this possibility could not be ruled out, even though the relatively slight tendency to reaction of the chlorine contained in chloroprene argued against it. In any case, it did not seem possible, without further investigation, to answer this question. In the tests made by the author for this reason, a comparison was instituted between carbontetrachloride and chloroprene under similar conditions. Several methods for the determination of phosgene have been proposed. Many of them are practical quick methods, important in view of the use of phosgene as a war gas. In many cases, however, these methods are not very sensitive and therefore not well suited for careful analysis. The author in his

tests has adopted especially the so-called aniline method. It is based on the development of diphenylcarbamide (which is sparingly soluble in aniline water) when the phosgene reacts with the aniline:

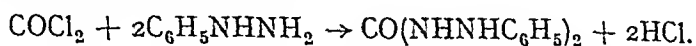


By previously saturating the aniline solution with diphenylcarbamide and afterwards filtering to a clear solution, this reaction will be very sensitive. When the reaction is disturbed by hydrochloric acid as well as by free chlorine, it is necessary to free the gas mixture from those substances. This was effected by the expedient that the gas, before absorption in the aniline water, was passed through wash-bottles containing mercury. The heating of the carbontetrachloride and the chloroprene was effected by immersing a glowing glass rod repeatedly into a beaker containing the respective substances. With carbontetrachloride the author obtained a greyish-white precipitate, the melting point of which was found to be  $237^\circ \text{C}$ , which tallies well with that of the diphenylcarbamide, which is  $236^\circ \text{C}$ . On the other hand, no precipitate was produced with chloroprene, whether non-stabilized or stabilized with pyrocatechin. After addition of pyrocatechin to the carbontetrachloride in the same amount as that used in the stabilization of the chloroprene, a precipitate of diphenylcarbamide was still obtained, which would indicate that this stabilizer in itself does not prevent the development of phosgene.

In radiation with ultraviolet light, the author used an ultraviolet lamp of 120 W, provided with a reflector permitting a good concentration of the rays. The source of light was placed ca. 7 cm over the surface of the liquid, and the radiation proceeded for several hours. The gases produced, as in the preceding test, were first sucked through wash-bottles containing mercury and were afterwards passed through aniline water saturated with diphenylcarbamide. In tests with carbontetrachloride a precipitate of diphenylcarbamide was produced also in this case, though in a smaller amount than on the previous occasion. On the other hand, no precipitate was obtained with chloroprene.

In some tests the author adopted a method for the detection of phosgene that had been proposed by ANGER and WANG (1938). It is based on the fact that phosgene with phenylhydrazine

forms diphenylcarbaid, which with cupric salts produces compounds with an intense violet colour:



This reaction, according to the said authors, is very sensitive and responds to amounts down to 0.5  $\gamma$  phosgene. Heating with a glowing glass rod as well as ultraviolet radiation were tried also with this method. With carbontetrachloride the characteristic colour showed up beautifully, whereas it was in no case obtained with chloroprene.

These tests have shown that phosgene is formed by carbontetrachloride, but not by chloroprene under the given conditions. TANDBERG and others had previously found similar experimental conditions to be very favourable for the formation of phosgene from carbontetrachloride as well as from several other chlorinated hydrocarbons. As it does not seem possible to show factors which could be more favourable for the development of phosgene from chloroprene, it may be stated, with the greatest probability, that we need not reckon with the development of phosgene from chloroprene, either in laboratory tests or in its use for industrial purposes.

## Studies on Animals.

### CHAPTER V.

## THE EFFECT OF THE CHLOROPRENE ON THE MORTALITY.

### A. Subcutaneous injection.

By using a finely graduated injection syringe, it was found possible with sufficient precision to administer amounts down to 0.000125 ml per g of the body-weight at weights of about 300 g. Pure chloroprene and stabilized oxidized chloroprene could be tested in this way, whereas the polymeric forms could scarcely be injected, especially on account of their high viscosity.

From a practical point of view, it was considered important to ascertain whether the toxic properties of chloroprene were changed by oxidation. It must in fact be expected that the chloroprene to which the workers are exposed in the factories had in many cases undergone oxidation processes. In this form of administration, the author therefore tested two different qualities of chloroprene.

The author has had the advantage of procuring the chloroprene in sealed ampullae from the laboratory of the Swedish Rubber Research Institute at Uppsala. The basic material for the other kinds of chloroprene tested by the author consisted of chloroprene from the factories, which had been stabilized there with pyrocatechin or thioldiphenylamine. This chloroprene was distilled by the author in an ordinary distillation apparatus, with water cooling. The author took the fraction that had distilled over at 59°.4 C, but always separated the first amount from the distillate, which otherwise as a rule would have been cloudy. Stabilization was then again made with pyrocatechin. Before tests were made with chloroprene that had been stored for some length of time, it was always first tested for the occurrence of polymers by the addition of alcohol. At the

least sign of precipitation, it was considered that it did not satisfy the requirements for non-polymerized chloroprene. The same rule was applied if the chloroprene had assumed any colour, or if its characteristic smell had changed.

The author studied the toxicity (1) of chloroprene which, after distillation, had been kept in sealed glass ampullae in nitrogen atmosphere and (2) of chloroprene which, after distillation, had been stabilized with pyrocatechin and had then been kept without shelter from air for some days. The first-mentioned non-oxidized chloroprene was injected immediately after the ampullae had been opened, but not until it had been stabilized with pyrocatechin. For a satisfactory stabilization, one need merely add ca. 0.5 g pyrocatechin to 100 g chloroprene. It having been found that injection of a 0.5 per cent aqueous solution of pyrocatechin in amounts corresponding to the presence of that substance in the injected chloroprene had no toxic effects on the animals, it was considered probable that in tests of the pyrocatechin-stabilized chloroprene the effect on the animals must be attributed solely to the chloroprene.

The tests were made on white rats of a strain that had been fed on the same full diet for a considerable length of time. The injections were made as uniformly as possible in regard to place, depth and rate. The author used 20 animals for each concentration. The minimum amount injected was 0.000125 ml per g body-

Table II. Mortality after sub-

Dosage ml per gram body-weight	Non-oxidized chloroprene					
	Mortality within 2 days			Mortality within 7 days		
	Number of 20	Deduced value*		Number of 20	Deduced value	
			per cent			per cent
0.000125	0/20	0/85	0	0/20	0/73	0
0.00025	3/20	3/68	4.4	4/20	4/57	7.0
0.0005	7/20	10/58	17.2	8/20	12/49	24.5
0.001	10/20	20/55	36.4	12/20	24/49	49.0
0.002	11/20	31/56	55.3	12/20	36/53	67.9
0.004	11/20	42/58	72.4	16/20	52/61	85.2
0.008	13/20	55/63	88.7	15/20	67/72	93.0

\* This value is estimated according to BEHRENS. (See BURN, 1937.)

weight. The dosages were doubled for each new series. The maximum dose was 0.008 ml per g body-weight. The animals were observed for 7 days.

The results from these tests are shown in table II and in Fig. 2 and 3.

It is evident that the toxicity of the two tested qualities are considerably different. The difference is more pronounced in the observations made after 2 days than in these made after 7 days. As regards the first-mentioned observation period  $DL_{50}$  of non-oxidized chloroprene showed to be some more than 0.002 ml per g body-weight, whereas  $DL_{50}$  of oxidized chloroprene amounted to 0.0005 ml. Thus it was found a difference of about 0.0015 ml between the dosages that killed 50 per cent of the animals, that is that oxidized chloroprene is about 4 times as toxic as non-oxidized.

After 7 days  $DL_{50}$  of non-oxidized chloroprene, however, was found to be 0.001 ml per g of body-weight, whereas with oxidized chloroprene the value was 0.0005 ml which is the same as in the observation after 2 days. The difference of  $DL_{50}$  between oxidized and non-oxidized chloroprene was now merely 0.0005 ml.

In the tests with the oxidized chloroprene 47 animals survived the whole period of observation, whereas with the non-oxidized chloroprene 73 animals survived longer than that period. The mean-value for the time that the animals had survived after injection of the respective amounts, with a single exception, was distinctly

cutaneous injection into rats.

Oxidized chloroprene					
Mortality within 2 days			Mortality within 7 days		
Number of 20	Deduced value		Number of 20	Deduced value	
		per cent			per cent
0/20	0/53	0	0/20	0/47	0
6/20	6/37	16.2	6/20	6/33	18.2
8/20	14/31	45.2	9/20	15/28	53.6
18/20	32/37	86.5	19/20	34/36	94.4
19/20	51/54	95.4	19/20	53/54	98.1
18/20	69/71	97.2	20/20	73/73	100.0
20/20	89/89	100.0	20/20	93/93	100.0

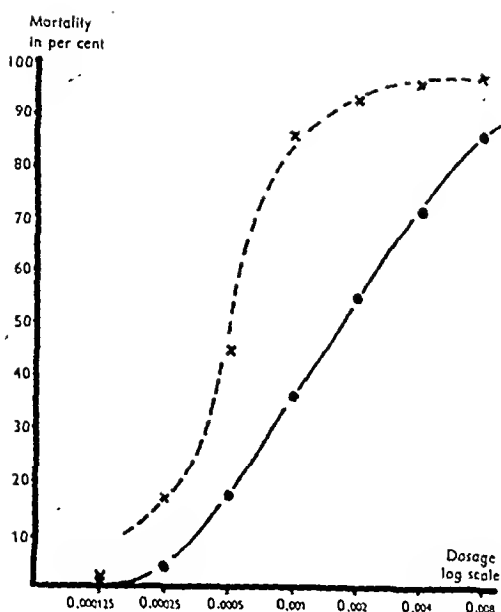


Fig. 2. — Mortality within two days after subcutaneous injection into rats  
 ● — ● non-oxidized chloroprene  
 x — — — x oxidized chloroprene

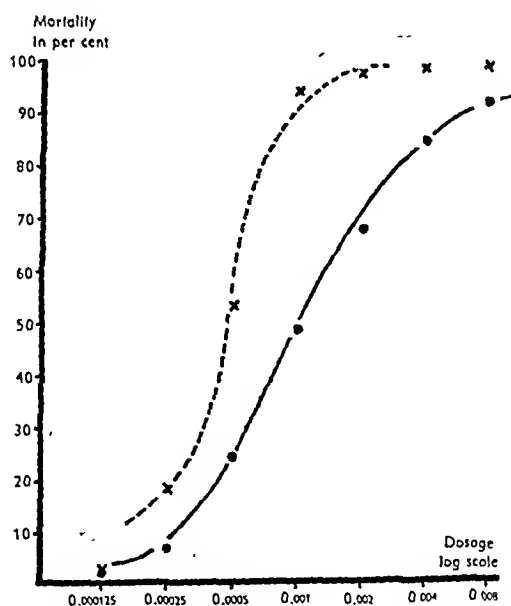


Fig. 3. — Mortality within seven days after subcutaneous injection into rats  
 ● — ● non-oxidized chloroprene  
 x — — — x oxidized chloroprene

lower for the oxidized than for the non-oxidized chloroprene, and the different "time of survival" were better grouped around their means in the former than in the latter kind of chloroprene.

In subcutaneous injection into rats v. OETTINGEN estimated M.F.D. to be 0.02 ml per g body-weight. Even taken into account that v. OETTINGEN and the present author have not computed the fatal dosage in the same manner the difference between the values is considerably marked. The discrepancies may, of course, be due to many different factors. It is generally known that different strains of the same kind of animal as well as differences in the diet may affect the results. The author's toxicity tests have also shown that different results are obtained from oxidized and non-oxidized chloroprene, which indicates the importance of precisely stating the kind of chloroprene to be tested.

### B. Inhalation.

In the inhalation tests, the author caused pressure air to pass through a gasometer and a manometer to a glass flask on the bottom of which an evaporation vessel with chloroprene had been placed. From the glass flask, the chloroprene-containing air was then transferred to the cage where the animals were exposed. By reading the amount of air that had passed and computing the loss of weight in the evaporation vessel, the concentration of chloroprene in the inspired air was calculated. (See Fig. 4.)

In these tests, it was found necessary to use only ground-in glass stoppers for the flasks and, as far as possible, to replace rubber tubes by glass tubes, as otherwise losses of chloroprene resulted. The author empirically found a suitable size of the evaporation vessel and was then able to obtain the evaporation intended by regulating the velocity of the pressure air. When a test was extended over several hours, the author supplied chloroprene during the course of the test, in order to avoid appreciable differences of the level in the evaporation vessel and thus also changes in the rate of evaporation.

In view of the practical importance of this whole investigation, it was considered to be of quite special interest to study the effects on animals after *inhalation of small amounts* of chloroprene for a considerable length of time. The concentrations of chloroprene in these tests were adjusted so as to be representative of the



chloroprene concentrations found in analysis of the air within the factory premises where chloroprene occurred in largest amount. Only in exceptional cases did these concentrations exceed 1.2 mg per litre of air; on the other hand, smaller amounts than 0.2 mg per litre of air were rarely found. In the animal experiments,

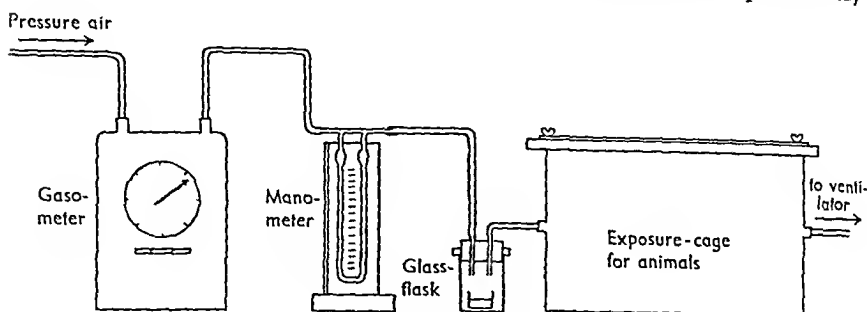


Fig. 4. — Apparatus for the inhalation tests.

these two concentrations were tested for 5 months, with a daily exposure of 8 hours. It was found possible, with the above indicated inhalation technique, to keep these concentrations practically constant, with merely minute fluctuations of less than  $\pm 0.03$  mg per litre of air.

In order that the chloroprene, from a toxic point of view, should be as uniform as possible, it was necessary daily to use freshly distilled and stabilized chloroprene. This was also essential in order to keep the evaporation rate practically constant. For each concentration, the author used 10 adult male rats, besides which he had 3 control animals for each series. During the daily exposure the animals did not receive any food or drink: they were not supplied with food and drink until they had been transferred, after the exposure, to the cages where they were usually fed. This applied also to the controls. At the end of each week the weight of the animals was controlled, besides determinations of the hemoglobin as well as counts of the red and white cells. The determinations were always made after the termination of the exposure for the day. (As for the blood-values, see Ch. VIII A.)

Moreover, the urine was weekly examined, by Schlesinger's test, for the occurrence of urobilin.

Particular interest attaches to the group exposed to a concentration of 1.2 mg per litre of air. These rats were rather markedly affected after the first exposures. They became sluggish and

apathetic, consuming but little food and drink during the first 10—14 days after the start of the experiment. This diminished ingestion of food manifested itself also in a considerable decrease of weight, particularly marked during the first week. After about a fortnight, however, the animals' appetite improved, and their need of food and drink seemed to be, broadly speaking, the same as that of the controls. The continuous loss of weight (see Fig. 8, p. 52) should not therefore be attributed chiefly to lack of sufficient food, but mainly to a toxic effect of the chloroprene. During the course of the test, 5 rats altogether died, one in the 6th week, two in the 9th and two in the 13th week.

In view of the usually very pronounced degenerative changes that occur in the liver after exposure to chloroprene (see Ch. XI), one might expect to find urobilin in the urine as an indication of a functional disturbance of the liver. The presence of that pigment in the urine was in fact observed by v. OETTINGEN in a number of cases. The present author, however, found, that urobilin occurred in his material comparatively seldom and very irregularly. Only in six out of the ten animals in this series could urobilin be detected. In one of the animals Schlesinger's reaction happened to be positive in the 7th week, but otherwise urobilin did not occur until the 8th and following weeks. The reaction as a rule was relatively feeble, and even in some cases with a rather marked positive reaction during one week the reaction during the following week might be negative, afterwards again showing the presence of urobilin. It should be mentioned here that three out of the five animals that died did not show a positive Schlesinger reaction on any occasion, and that the two others did not show urobilin to any greater extent than had occurred in four out of the five survivors. This indicates that, even after exposure to a relatively high chloroprene concentration, the liver may nevertheless continue to function sufficiently well to prevent any appreciable disturbance of the urobilin circulation.

In the series where 10 rats had been exposed to 0.2 mg chloroprene per litre of air under otherwise the same conditions as in the preceding group, none of them died in the course of the whole experimental period. These rats, moreover, were much less affected than those in the other series. Only during the first few days could some apathy in the rats be noticed, as well as some

loss of appetite. It was not, however, so marked that any loss in weight could be recorded. Nor did the rats lose weight during the further exposure, and their consumption of food was quite equal to that of the control animals. In examining the urine for urobilin, the author did not find a positive Schlesinger reaction in any case.

v. OETTINGEN et al. exposed 10 rats and 20 mice for three months to the same concentration as the author had used in the last described series. They do not, however, record the length of the daily exposure time, nor do they state whether newly distilled chloroprene had been used. The variations in the concentrations from 0.1 to 0.3 mg chloroprene per litre of air are indicative of varying evaporation rates, which may have been due to chemical changes in the chloroprene. One of the ten rats died in the 5th week and another one in the 7th. Among the mice the mortality was considerably larger, totalling 9 deaths in the course of 3 months.

The effect of chloroprene in *inhalation of the higher concentrations* has been considered, in view of the practical purpose of the investigation, to be of minor interest. In three experimental series the author, however, has studied the mortality in rats that had been exposed for 8 hours to chloroprene concentrations of 3.5, 10 and 17.5 mg per litre of air. Each series consisted of 10 animals. In these tests freshly distilled, pyrocatechin-stabilized chloroprene was used, and the same exposure technique as in the above described long-time experiments was adopted. The animals were followed up to 48 hours after the beginning of the exposure.

It appeared from these tests that a concentration of 17.5 mg of chloroprene per litre of air killed all the animals within 48 hours. As, however, 60 per cent of the animals died at a concentration of 10 mg of chloroprene per litre of air, it seems probable that even a lower concentration than 17.5 mg would have caused a hundred per cent mortality. With the same method of administration and the same exposure time, v. OETTINGEN considers that the minimal fatal dose (MFD) for rats lies between 15 and 21 mg chloroprene per litre of air. As these figures are based on very small series with very unequal results, the "security" does not seem to be particularly convincing. As v. OETTINGEN has not stated the precise chloroprene quality tested, a direct comparison with the present author's own mortality figures can scarcely be made.

## CHAPTER VI.

### THE EFFECT OF THE CHLOROPRENE ON THE CIRCULATION AND RESPIRATION.

In a series of experiments on rabbits and cats, v. OETTINGEN et al. studied the effects of chloroprene on the circulation and respiration during inhalation. The chloroprene was supplied to the animals through a tracheal cannula, and the blood pressure was estimated at the carotic artery. The blood pressure and respiration were recorded in the usual way by a cynograph. The said authors found a similar course in all their experiments. Minor irregularities in pressure and respiration were noted immediately after the administration of chloroprene, but then a continuous fall of the arterial pressure set in as well as a somewhat slowed respiration. The latter, however, was still almost intact even towards the termination of the tests, when the arterial pressure had reached its minimum. In order to ascertain the cause of the observed fall of blood pressure, the authors studied the effect of chloroprene on isolated frog's heart according to STRAUB. In these tests, the heart was exposed "to vapors of chloroprene and even to liquid chloroprene by dropping it on the organ." No effect on the contractility of the heart was, however, noted.

The possibility that chloroprene might have a central point of attack and affect the blood pressure via medullary centres was studied in experiments on cats. v. OETTINGEN et al. caused ammonia to be insufflated into the animals' nostrils, in order reflexively to stimulate centres in the medulla. Chloroprene did not seem to reduce the effect of such activation, and the authors accordingly inferred that the substance had no depressant effect on medullary centres.

Another question raised by the said authors was whether the fall of the blood pressure might be due to a direct effect of chloro-

prene on the peripheral vessels. This matter was investigated "by perfusing the hind legs of frogs" in accordance with the method of TRENDELENBURG. It was found that, even with very dilute solutions of chloroprene in water, a distinct vasoconstrictory effect was obtained.

The experiments made thus did not serve to explain the fall of the blood pressure. The observed effect on the peripheral vessels was, of course, particularly noteworthy and apparently contradictory. However, in view of the hyperemia observed in the abdominal organs in other experiments with chloroprene, they considered it probable that the fall of the blood pressure was attributable to a dilatation of the vessels in the splanchnic region.

The question as to the mechanism in the lowering effect of chloroprene on the blood pressure seemed, however, to the present author scarcely to have been satisfactorily settled with this supposition. It seemed therefore desirable to endeavour to throw light on this problem by further investigations. The author made experiments on rabbits and cats *in vivo*, as well as on isolated rabbit and frog hearts in accordance with the technique of LANGENDORF and STRAUB. (See GADDUM, 1948.) The first-mentioned experiments were made on some thirty animals, most of which consisted of rabbits. The animals were operated under urethane narcosis. As a rule, it was found sufficient to inject about 3 ml per kg of the body-weight of a 25 per cent aqueous solution of urethane. In these experiments, the author tested the effect of freshly distilled, pyrocatechin-stabilized chloroprene as well as of chloroprene which, after distillation and with and without stabilizer had been kept for a longer or shorter space of time. The chloroprene was supplied to the animals by inhalation through a tracheal cannula. The chloroprene was not exactly dosed, but the experimental conditions were arranged so that, on comparison between different qualities of chloroprene, practically the same amounts were supplied. The blood pressure was estimated firstly at the carotic artery and secondly at the right auricle through a cannula introduced from the jugular vein. The respiration was recorded from the tracheal cannula by means of a Marey's capsule. In all these tests, the author took electrocardiograms before the exposure to chloroprene and on repeated occasions while the exposure was proceeding.

As regards the respiration, the author, in certain respects, was

able to verify the observations of v. OETTINGEN. In most cases, in immediate connection with the administration of chloroprene, a brief apnea or a certain irregularity in the respiration set in. It was evident that these changes were most marked when the animals were exposed to chloroprene which had been oxidized, and less pronounced when freshly distilled chloroprene was used.

The respiratory condition may be regarded as a reflex apnea, often occurring on inhalation of irritant vapours. The more powerful effect of the oxydized chloroprene in this respect is presumably due to the formation of acid oxidation products, and to the  $\beta$ -polymer, the development of which cannot be completely prevented despite the pyrocatechin stabilization, and which has a pungent turpentine-like smell. In some tests the author observed a slowed rate of respiration; this, however, was not a constant effect, being

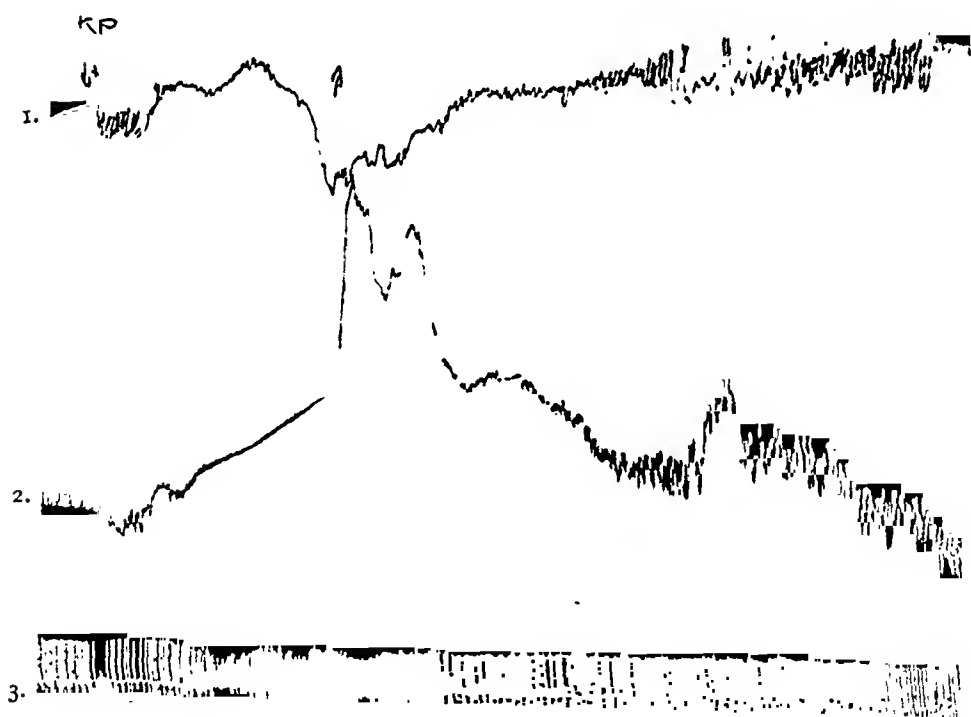


Fig. 5. — Tracing of blood pressure and respiration in cat at inhalation tests of chloroprene.

1. Blood pressure at the carotic artery
2. Blood pressure at the right auricle
3. Respiration.
- ↓ Chloroprene is administered
- ↑ Chloroprene is suspended

absent in many cases. In all the tests, the respiration still continued for a short time after the heart's action had ceased.

The variations in the blood pressure showed a similar course in all the tests. After minor, transient irregularities in connection with the above-described respiratory disturbances when chloroprene was first supplied, the arterial pressure fell continuously, whilst the pressure in the right auricle rose. According to the amount supplied, these changes occurred with different rapidity and with different intensity. Finally, on the continued administration of chloroprene arrest of the heart set in, the heart stopping at diastole. No differences in the variation of blood pressure with different kinds of chloroprene could be observed. As regards the electrocardiograms, no pathological changes could in any case be observed on the curves.

Especially in view of the rise of pressure in the right auricle, there were indications that the changes in the blood pressure were attributable to a cardiac insufficiency. It accordingly seemed desirable to study the effects of chloroprene on the isolated heart. This, as above indicated, was done by the author (1) on rabbit's heart and (2) on frog's heart. In the first-mentioned experiments the animals were operated under urethane narcosis. When the heart had been carefully washed free from blood in luke-warm Ringer's solution, arrangements were made for tests according to the technique of LANGENDORF. The muscle contractions from the ventricular part of the heart were recorded by a cymograph. The experimental conditions were arranged so that the heart could be supplied either with pure Tyrode's solution<sup>1</sup> or chloroprene dissolved therein. The solutions were kept at a temperature of 38° C and were continuously bubbled through with oxygen. In order to obtain exact chloroprene concentrations, freshly distilled, pyrocatechin-stabilized chloroprene was first dissolved in aqua destillata by shaking for 15—20 hours at 20° C. The author has shown (see Ch. IV) that in this process about 0.05 ml of chloroprene is dissolved in 100 ml of water. On the basis of this solution, the required concentrations of chloroprene in the Tyrode's solution could be obtained. The author tested several concentrations with chloroprene amounting as a maximum to 0.5 ml and as a minimum to 0.001 ml, per 1,000 ml Tyrode's solution.

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<sup>1</sup> The Tyrode's solution was of the following mixture:—0.8 % NaCl, 0.02 % KCl, 0.02 % CaCl<sub>2</sub>, 0.02 % MgCl<sub>2</sub> · 6H<sub>2</sub>O, 0.1 % NaHCO<sub>3</sub>, 0.005 % NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O and 0.1 % glucose.

The effect on the contractions of the heart muscles was very marked at the higher chloroprene concentrations, and the amplitude of the responses was reduced to about one-tenth of that when Tyrode's solution solely was used. According as weaker solutions were employed, the effect was reduced, but even at concentrations of 0.002—0.003:1,000 it was still measurable. No change of the cardiac rhythm, on the other hand, could be observed either at high

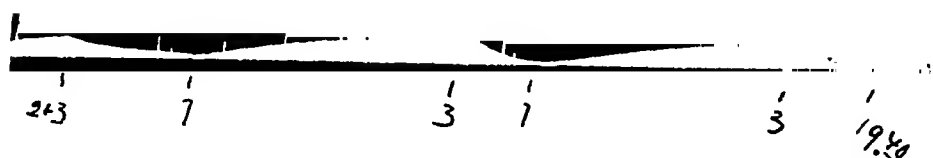


Fig. 6. — Test with chloroprene on isolated rabbit's heart. Contractions of left ventricle.

1. Only Tyrode's solution.
3. 0.01 ml chloroprene per 1,000 ml. Tyrode's solution

or low concentrations. In connection with these tests, the author compared the coronary circulation in the case of chloroprene solutions and Tyrode's solution solely by computing the amount of solution that passed through the heart during equal units of time, but no effect on the coronary circulation could be found.

In tests with chloroprene on frogs' hearts according to STRAUB's technique, the author proceeded, as in the preceding tests, from a saturated aqueous solution of freshly distilled pyrocatechin-stabilized chloroprene. The latter was dropped direct into the



cannula (rather wide at the top), filled with Ringer's<sup>1</sup> solution, that had been introduced into the aorta. The cannula had a capacity of 3.8 ml and, by reckoning the number of drops of chloroprene solution supplied, an approximate estimate of the chloroprene concentration could be obtained. The heart contractions were recorded essentially in the same way as in the LANGENDORF test. The frog's heart was found to react similarly as the rabbit's heart. At higher concentrations it responded very quickly with a considerably reduced amplitude of the excursions. In tests with weaker concentrations the effect was less marked, but could be distinctly recorded even at a concentration (approximate) of 0.01 ml chloroprene per 1,000 ml Ringer's solution. No effect on the cardiac rhythm was noticeable.

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<sup>1</sup> The Ringer's solution for frog's heart was of the following mixture:—  
0.01 %  $\text{NaHCO}_3$ , 0.01 %  $\text{CaCl}_2$ , 0.0075 %  $\text{KCl}$ , 0.6 %  $\text{NaCl}$ .

## CHAPTER VII.

### THE EFFECT OF THE CHLOROPRENE ON THE LUNGS.

In connection with the estimation of  $DL_{50}$  on subcutaneous injection of chloroprene into rats (see Ch. V A), the author determined the weights of the lungs in every other animal in both series, i. e. those series who had received (1) non-oxidized and (2) oxidized chloroprene. Thus the two series comprised 70 animals, consisting of groups of ten for each concentration. Those that survived 7 days (168 hours) after the respective injections were then killed by a blow on the nape. In this way, a relatively large number of rats from the groups injected with small amounts of chloroprene were killed after 7 days, whereas almost all the rats that had received larger amounts died spontaneously within this time. Dissection and removal of the lungs was carried out uniformly for all the animals. The weight of the lungs in percentage of the body-weight was computed. This computation was based on the body-weight at the beginning of the tests, it having been found that, after the injections, these weights were considerably affected by individual variations in the consumption of food. When the means of the percentages in each group had been computed for the two series, these percentages were recorded in the diagram in next page.

The most striking fact in this comparison is the marked difference in effect between oxidized and non-oxidized chloroprene at the higher dosages. The lung weights in fact then show a considerable increase after administration of oxidized chloroprene, which increase had no correspondence in tests with non-oxidized chloroprene. Another noteworthy fact is the uniform variation in the weights of the lungs in the two series after administration of small amounts of chloroprene, up to 0.0005 ml per g body-weight. It

seems rather difficult to find any convincing explanation of the actual variations whilst the correspondence between these series must be viewed in the light of the fact that the thus recorded lung-weights in both series, as to about 80 per cent for each, refer to animals that had survived the observation time and had afterwards been killed. The toxic effect of the amounts injected had thus been relatively slight; it was only at higher dosages, from 0.001 ml per g

Weight of lungs  
in percent of body weight

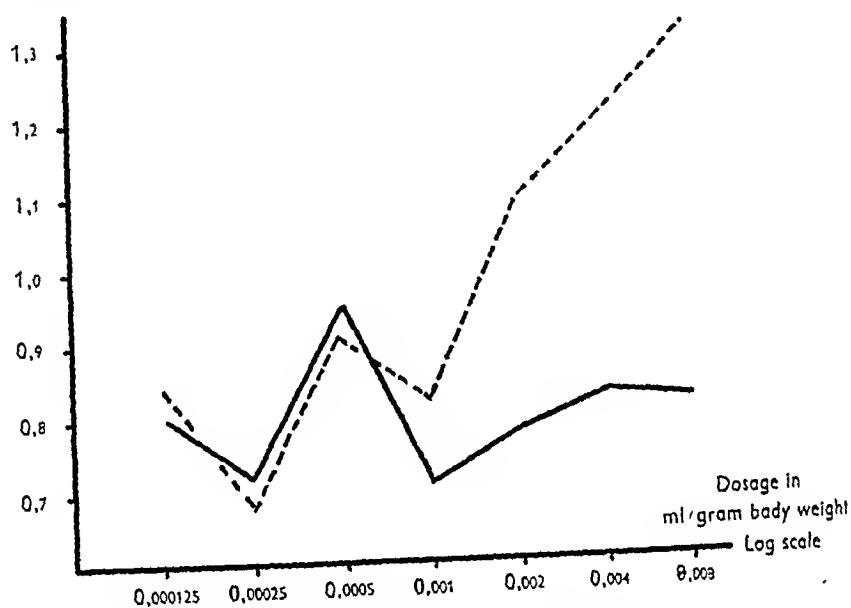


Fig. 7. — The weight of lungs after injection of chloroprene into rats.  
—— non-oxidized chloroprene  
----- oxidized chloroprene

body-weight and upwards, that the toxic effect became more marked, as indicated by the fact that merely 12 and 5 per cent, respectively, of the animals from the groups injected with non-oxidized and oxidized chloroprene, respectively, survived. Moreover, it was only at the higher dosages that the different effect on the weights of the lungs was manifested. It was also evident even from the gross appearance of the lungs of animals that had received at most 0.0005 ml chloroprene per g body-weight, that the lungs as a rule showed no, or very slight, deviation from the normal, whether injected with

oxidized or non-oxidized chloroprene. Nor did the microscopic examination of these lungs show any noteworthy pathological findings. This was, broadly speaking, the case also at the higher dosages of non-oxidized chloroprene. In certain cases, however, slight hyperemia and a small amount of blood in some of the bronchi was observed.

The postmortem findings in the lungs of the animals injected with oxidized chloroprene in dosages from 0.0005 ml and upwards were quite different. The lungs of these animals were larger, with some pale-red parts, whilst other parts were darker in colour. On dissection, edematous fluid issued from some of the lungs, whilst other showed signs of marked hyperemia. The microscopic examination likewise showed hyperemia, with occasional hemorrhages and, in many cases, a marked edema. In most of the cases emphysema was observed here and there. Within minor parts of certain lungs the alveoli were distended and thin-walled.

In a minor material consisting of 15 rats, inclusive of 5 controls, the author studied the effect on the lungs after inhalation of chloroprene. The animals were exposed for 5 hours in air mingled with stabilized, oxidized chloroprene. The concentration in these tests was about 17 mg chloroprene per litre of air. Rather soon after the beginning of the exposure, the rats showed an accelerated respiration rate and towards the end of the experiment a rather marked dyspnea. At the end of the exposure period all the rats were killed with a blow on the nape, whereupon the lungs were weighed, their weights being computed in percentage of the body-weight. The mean of these percentages for the 5 controls was  $0.88 \pm 0.05$ , and the corresponding value for the 10 experimental animals  $1.64 \pm 0.06$ . Thus, a rather considerable increase in the weight of the lungs, larger than after the subcutaneous injection of chloroprene, had occurred. On macroscopic inspection, these lungs also appeared to be more edematous, in some cases with a rather copious foamy fluid on incision of the lung tissue. A fluid of similar appearance was found also in the bronchi. The bronchial mucous membranes as a rule were rather markedly reddened. The microscopic examination in all cases showed a pronounced edema as well as stasis.

The macroscopic as well as the microscopic examination showed that the increase in the weights of the lungs in both experiments

were attributable partly to stasis with hyperemia in the pulmonary vessels, partly to edema, with an efflux of fluid into the alveoli. In the inhalation tests the lungs appeared to be more edematous than after subcutaneous injection of chloroprene, where the hyperemia of stasis was more marked. Whether the edema was to be regarded as due solely to stasis or partly also as caused by a direct local effect of chloroprene on the lungs could not be ascertained with certainty by these tests. It may be presumed, however, that this latter factor had a bearing on the occurrence of edema after inhalation, where the edema was strikingly marked in comparison with the stasis. In connection with the hematocrit tests (see Ch. VIII B), where indeed the experimental conditions were different, the author has also shown that the loss of plasma in itself presumably suffices to account for such an increase in the weight of the lungs as had occurred in these tests.

## CHAPTER VIII.

### THE EFFECT OF THE CHLOROPRENE ON THE BLOOD.

#### A. Changes in the hemoglobin value and in the number of erythrocytes and leucocytes.

As mentioned above in connection with the chronic inhalation tests (see Ch. V B) on 13 rats, incl. 3 controls, the author estimated the hemoglobin values and the number of red and white cells every week during the courses of five months.

The blood samples were taken from the tail, and the hemoglobin determination was made with a standardized Autenrieth's colorimeter.

The hemoglobin values as well as the red cell counts during the first stage of the experiment showed a distinct rise in the exposed animals. It seems difficult to account for this rise otherwise than as a "drying" effect. As the rats during the first and second week consumed very small amounts of food and drink, such a mechanism is quite conceivable. A contributory factor may also have been a certain loss of fluids in the blood-stream owing to the development of pulmonary edema. The animals that died during the course of the test in fact showed signs of such a pulmonary condition. Elsewhere in this paper (see Ch. VII), the author has shown that oxidized chloroprene in particular, even in small concentrations, causes pulmonary edema, and that the hematocrit values rise after exposure to chloroprene (see Ch. VIII B). The increase in the hemoglobin and red cells rather soon passed over, and was then followed by a decrease, which continued during the whole exposure period. This decrease affected the hemoglobin to the same extent as the number of red cells, and there were no marked changes in the colour-index.

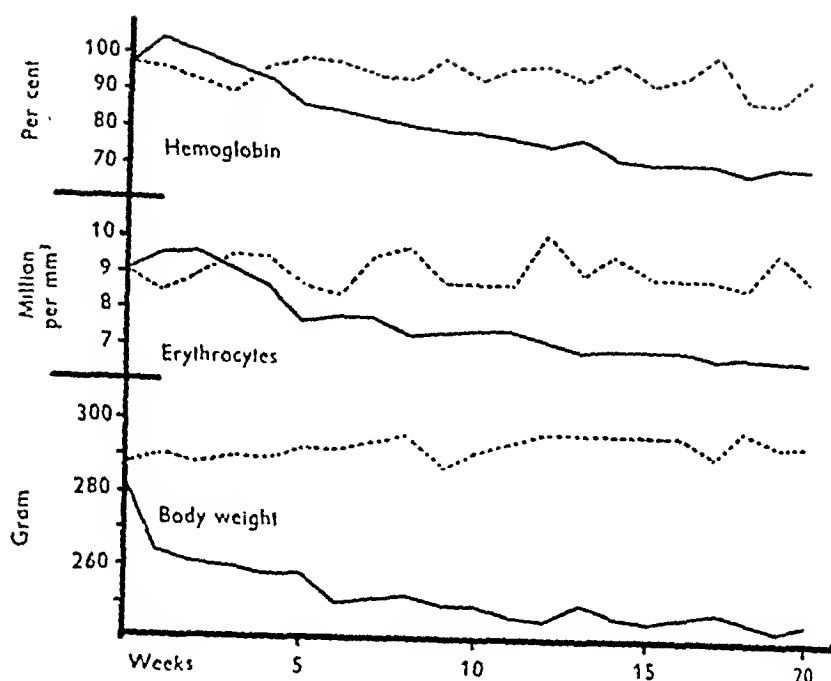


Fig. 8. — Hemoglobin value and number of erythrocytes and body-weight of rats.

Exposure: 1.2 mg. chloroprene per litre of air for 8 hours daily in 20 weeks.

— exposed animals

..... control animals

. Thorough investigations into the changes in the composition of the blood, including tests in rats exposed to chloroprene, have been made by v. OETTINGEN. As regards the hemoglobin and erythrocyte values, however, they were studied mainly in short tests, and no certain changes in those values could then be established. We find in the literature comparatively few detailed observations into blood changes in animals caused by other aliphatic chlorinated hydrocarbons closely related to chloroprene. LEHMANN and SCHMIDT-KEHL (1936), for example, followed the hemoglobin and erythrocyte values in rabbits and cats in lengthy tests with dichloromethane, tetrachlormethane, transdichlorethylene and trichlorethylene. These tests yielded rather varying results, with both rising and falling values, during the time in which the exposure proceeded. Trichlorethylene produced a distinct decrease of the hemoglobin values in rabbits, whilst the number of red cells showed

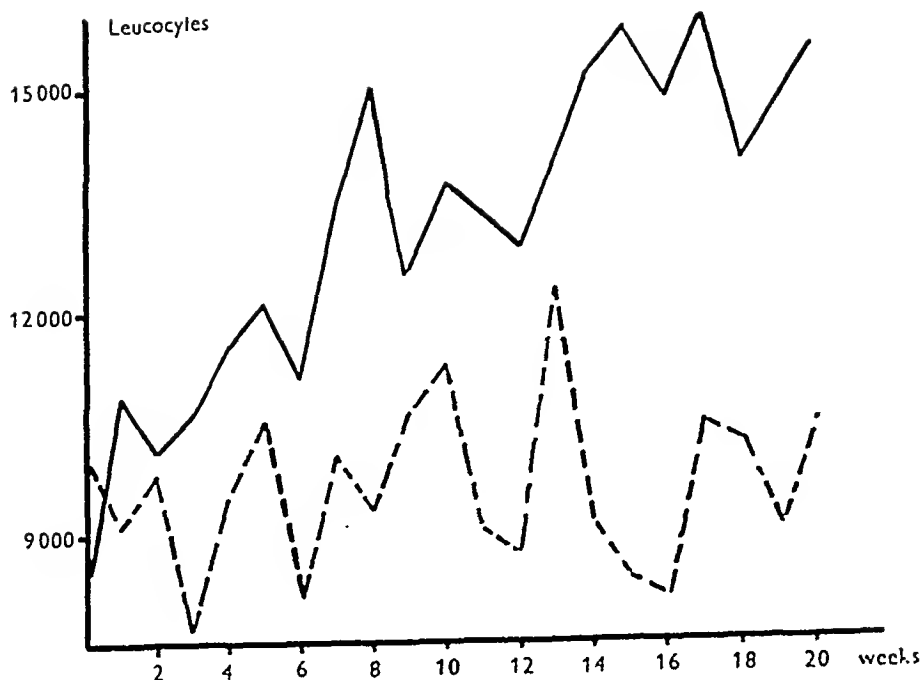


Fig. 9. — Number of leucocytes of rats.  
 Exposure: 1.2 mg. chloroprene per litre of air for 8 hours daily in 20 weeks.  
 ——— exposed animals  
 - - - - - control animals

a slight rise. These tests and reports from other authors have not given any convincing evidence regarding changes in the hemoglobin and erythrocyte values on exposure to the above-mentioned substances. Most of these tests were made on a rather small number of animals, and the latter were followed merely for a rather short space of time.

The interpretation of changes in the leucocyte number in animals often presents great difficulties. Even normally, the leucocyte values are subject to very marked variations. According to GRIFFITH and FARRIS (1942), the mean number is about 9,000 in adult rats, with normal limits between 6,000 and 18,000. In observations that have to be carried on for a considerable length of time, it is usually impossible to avoid the animals being attacked by intercurrent infections, which affect the number of leucocytes. This is particularly the case in toxicological tests where the general condition of the animals is impaired. It is moreover known that other factors also,



such as emotional excitement or a struggle, even for a short time, may give rise to changes in the white-cell picture.

The present author followed the leucocyte number by regular taking of blood samples in the way described above with regard to hemoglobin and erythrocytes. The values varied considerably from time to time, but the animals exposed to chloroprene showed a rather marked continuous rise, which occurred to a much smaller extent in the controls. The differential blood-count was only carried out at some occasions and, though a relative lymphocytosis was observable, the author's material in this respect was too small to be significant. A leucocytosis was found by v. OETTINGEN after a single injection into rats; but in that material too, marked variations occurred during the observation time. In the above-mentioned investigation by LEHMANN the leucocytes showed an increase in number in most of the animals that survived the whole experimental period.

Also in the series of rats that was exposed to 0.2 mg per litre of air blood examinations were made to the same extent as in the preceding series. No certain tendency to a change in the blood values could be observed. In a few of the rats minor falls of the hemoglobin and erythrocyte values were recorded after some weeks, but they were quite temporary and, with one or two exceptions, were never found during two succeeding weeks. The leucocyte number was also remarkably constant in this series, and no certain deviation from the number in the control animals could be observed.

### **B. Changes in the plasma content.**

In connection with toxicological investigations after subcutaneous injection of chloroprene into rats, the author has shown that the weight of the lungs increased with rising dosages of oxidized chloroprene. This increase was manifested by advancing pulmonary edema according as the chloroprene dosage was raised. It seemed to be of interest to investigate whether the accumulation of fluid in the lungs could be recorded as changes in the hematocrit values. In order to study these conditions, the author determined the total erythrocyte volume in rats (1) before and (2) after the exposure to chloroprene.

The tests were made on 10 white rats weighing between 240 and 310 g. The blood samples were obtained by cuts in the tail. The author used a hematocrit pipette devised by VAN ALLEN (1925), which was found to be very serviceable for hematocrit determinations in animals. Contrary to VAN ALLEN the author used heparin as an anticoagulant, with which the pipettes were moistened and then allowed to dry. In the double determinations at first made, two pipettes being simultaneously filled, the differences in the values thus obtained were so slight that a single determination was considered quite sufficient. On the other hand, it was found necessary always to provide for a satisfactory spontaneous outflow of blood in order to obtain reliable values. The pipettes were centrifuged for 20 minutes at a speed of about 2,000 r.p.m. Further centrifuging did not change the corpuscle volume.

The animals were exposed to chloroprene which, after distillation, had been stabilized with pyrocatechin. By bubbling pressure air through this chloroprene, the author ensured that it was strongly oxidized before being supplied to the animals in the exposure cage. Determinations of the chloroprene concentration in the inspired air were not made in these tests, as their object was only to ascertain whether chloroprene affected the hematocrit value, but not to follow the variations of the latter at different concentrations of chloroprene.

Table III. The hematocrit values and plasma content of the blood in ten rats after exposure to chloroprene.

Hematocrit value		Plasma content in per cent		Difference in per cent of the initial value
Before exposure	After exposure	Before exposure	After exposure	
46	53	54	47	13.0
45	51	55	49	12.0
44	49	56	51	8.0
43	51	57	49	14.0
43	51	57	49	14.0
40	49	60	51	15.0
46	49	54	51	5.6
43	51	57	49	14.0
49	52	51	48	5.0
43	51	57	49	14.0
		55.8	49.3	11.6
		± 0.77	± 0.42	

By maintaining the same conditions for evaporation in all the tests, the chloroprene concentration was kept at about the same level. After first taking a normal value, the animals were exposed for 20 minutes, whereupon the hematocrit value was again determined.

The values found are recorded in table III. The figures show the readings on the hematocrit pipette. The content of plasma and the loss of plasma in percentage is also estimated.

It may be seen from this table that the fall in the plasma content of the blood averaged 11.6 per cent. Taken into account that the total blood volume in rats amounts to ca. 6.3 per cent of the body-weight (SCARBOROUGH, 1931) and that the amount of plasma in the exposed rats in the author's material averages 55.8 per cent of the blood volume, it will be found that the blood, owing to the exposure, loses ca. 1 g of plasma. In estimating the increase in the weight of the lungs after subcutaneous injection, the author found that at the maximum dosage it had increased by ca. 1.25 g, which thus well harmonizes with the estimated loss of plasma. In fact, the difference of ca. 0.25 g in all probability lies within the margin of error for these calculations, though it might in any case be expected that the loss of plasma should be somewhat less than the increase in the weight of the lungs, seeing that there is normally some resorption of fluid from the tissues into the blood.

### C. Changes in the oxygen content and the oxygen capacity.

Defective oxygenation of the arterial blood may be due to several different factors, which are to be found either on the "lung side" or on the "blood side." The author has shown that pulmonary disease occurred in rats after injection or inhalation of chloroprene (see Ch. VII), owing to the development of pulmonary edema. Even if this edema was of relatively moderate degree, it seemed to be of interest to find out whether it caused any disturbance in the oxygenation of the blood. Analogous conditions are known e. g. from LAQUEUR's and MAGNUS' (1921) investigations into the pathology of phosgene poisoning, in which, however, a much more marked pulmonary edema usually occurred. In view of the probability that when stabilized chloroprene is kept for a considerable length of time, peroxides are formed (KLIT, unpublished), it seemed

that a noxious effect on the blood was also conceivable. In particular, one might expect a formation of methemoglobin, analogous with the effect of several compounds with an oxidizing capacity, such as potassiumferricyanide, potassiumpermanganate, potassiumchlorate, etc.

With a view to the study of these problems, the author determined the oxygen content in the arterial blood of rats before and after exposure to stabilized and much oxidized chloroprene as well as the oxygen content and oxygen capacity in rabbits under the same conditions. It was in fact found difficult to obtain a sufficient amount of blood for both determinations in rats. The experimental series comprised 15 rats and 6 rabbits. The blood samples in all cases were taken from the carotic artery, which had been exposed under narcosis, after which a glass cannula had been introduced in the artery. As a narcotic, the author used a 20 per cent solution of ethylurethane, of which the rats received 0.5 ml per 100 g of the body-weight, and the rabbits 4—5 ml per kg of the body-weight, by subcutaneous injection. In computing the oxygen content, the blood was taken direct under paraffin oil and immediately analyzed in an ordinary Van Slyke apparatus for the determination of blood gases. In most cases double determinations were made, with good results in regard to correspondence. A maximal saturation of the blood with oxygen was effected by causing a strong current of air to sweep over a thin layer of the blood, which had been collected in a wide flask. As an anti-coagulant, the author used heparin. After first taking a blood sample by introducing the cannula into the carotic artery, the animals were exposed to chloroprene of the above indicated kind by putting them in a cage in which chloroprene had been allowed to evaporate. The chloroprene concentration was not precisely determined and varied from case to case. By observing the corneal reflex and the general relaxation, the author tried to obtain the same depth of narcosis. After the termination of the exposure, blood samples for renewed analyses were taken in the way above described.

The results of these determinations are shown by the following tables IV and V.

In the experiments on rats as well as those on rabbits, the oxygen content in all cases was lower after the exposure to chloroprene than before it, and more marked in the rats than in the rabbits.

A fall of the oxygen content in the arterial blood in connection

Table IV. The oxygen content of the arterial blood in rats before and after exposure to chloroprene.

Before exposure	After exposure	Difference in per cent of the initial value
19.77	17.69	10.5
20.58	16.79	18.4
22.78	13.26	41.8
18.13	14.02	22.3
20.19	14.50	28.2
18.15	14.55	19.8
21.30	19.47	8.6
20.35	17.17	15.6
18.74	16.73	10.7
23.04	18.99	17.6
19.69	16.08	18.3
22.28	19.32	13.3
21.84	19.34	11.4
23.54	20.32	13.7
21.92	20.04	8.7
20.82	17.22	17.3
± 0.45	± 0.60	

with narcosis has been observed by several investigators. THOMAS (1898) found this to be the case under ether narcosis, and similar observations have been made later, amongst others by PITT (1927). According to FUSS and DERRA (1930), the oxygen content in the arterial blood under ether narcosis is greatly dependent on the technique. Thus it was reduced under ether drop narcosis, but distinctly raised in ethyloxide narcosis. In a subsequent investigation by DERRA (1936), the latter found that the oxygen content was as a rule reduced in ether narcosis. In narylene narcosis the conditions varied, the values being now raised, now lowered, and this applied also to avertin narcosis. SCHAW, STEELE and LAMB (1937) found in dogs under ether narcosis a diminished saturation of the oxygen of the arterial blood, but, in general, a slight rise of the oxygen content, which was attributed to a simultaneously observed increase in the oxygen capacity of the blood. DERRA thinks it probable that a decrease of the oxygen content in the arterial blood under ether narcosis is due to a diminished oxygen tension in the alveolar air, whereas SCHAW, STEELE and LAMB consider this factor, generally speaking, to be an insufficient explanation. With reference to the investigations of VAN SLYKE, AUSTIN and CULLEN (1922) as well as those of CULLEN,

Table V. The oxygen content, oxygen capacity and oxygen saturation of the arterial blood in rabbits before and after exposure to chloroprene.

The oxygen content in the arterial blood			The oxygen capacity in the arterial blood			The oxygen saturation in the arterial blood	
Before exposure	After exposure	Difference in per cent of the initial value	Before exposure	After exposure	Difference in per cent of the initial value	Before exposure	After exposure
16.52	15.14	8.4	19.60	16.70	14.8	84.3	90.7
14.75	13.88	5.9	15.22	14.32	5.9	96.9	96.9
14.63	13.93	4.8	16.39	14.61	10.9	89.3	95.4
15.46	13.85	10.4	16.58	14.77	10.9	93.3	93.8
16.14	14.18	12.1	16.48	15.08	8.5	97.9	94.0
12.57	11.56	8.0	12.62	11.61	8.0	99.6	99.6
15.01	13.76	8.3	16.15	14.52	10.1	92.9	94.8

AUSTIN, KORNBLUM and ROBINSON (1923) on the acid-base equilibrium in the blood under ether narcosis, etc., SCHAW et al. attach considerable importance in this respect to the shifting of the pH towards the acid side, with the result that the dissociation of the hemoglobin is diminished.

As for the pulmonary edema as a possible cause of a reduction of the oxygen content in the arterial blood, this content, as previously indicated, is obviously dependent on the spread of the edema. LUNDSGAARD and VAN SLYKE (1923) point out that a relatively extensive obstruction involving up to two-thirds of the lung tissue, is required in order to cause any serious disturbances in the oxygenation of the blood.

If thus, in accounting for the reduction of the oxygen content in the arterial blood, no decisive importance can be attached, with any degree of certainty, to the pulmonary edema, there is another explanation that seems to be more reasonable. In the author's experiments on rabbits, it was noteworthy that the oxygen capacity of the blood fell after exposure to chloroprene. Changes in the oxygen capacity of the arterial blood in connection with narcosis have been observed by several authors (FUSS and DERRA, SCHAW et al.). Generally speaking, however, the oxygen capacity in such cases had increased, and often rather considerably. SCHAW et al. found a rise up to 18.7 per cent. Most investigators have explained this increase in the oxygen capacity of the blood as an

attempt of the organism to compensate an anoxemia by an increase of the hemoglobin.

In the author's experiments on rabbits, the percentage reduction of the oxygen capacity after the exposure to chloroprene was about 10 per cent, and the diminution of the oxygen content in the blood fully 8 per cent. As the computation of the degree of saturation for oxygen in the blood before and after the exposure to chloroprene showed practically corresponding values, the reduction of capacity seems to suffice to explain the fall in the oxygen content of the arterial blood.

As the author mentioned introductively, a fall of the oxygen capacity in the arterial blood on exposure to chloroprene seemed to be attributable primarily to a formation of methemoglobin. He investigated the occurrence of methemoglobin in the blood of rats that had been exposed to chloroprene of the same kind as that used in the above tests. The same kind of chloroprene was used also in experiments *in vitro*, the chloroprene being added direct to the blood. In these determinations, the author had access merely to a spectroscopic method of determination of small sensitivity, which gave no response until a methemoglobin concentration of 20—25 per cent had been reached. In the application of this method, no methemoglobin in the tests either *in vivo* or *in vitro* could be observed. But, as the reduction of the oxygen capacity in the above-reported experiments on rabbits in no case exceeded 15 per cent, it is not inconceivable that a formation of methemoglobin may nevertheless have occurred. Further investigations will be required in order to throw light of this matter.

#### D. The Coagulation of the Blood.

At autopsy of animals poisoned with chloroprene, v. OETTINGEN et al. frequently found infarcts and emboli in various organs. As it appeared possible to those authors that this might be an indication of the tendency of the blood to clot more readily after exposure to chloroprene, this problem was taken up for investigation. For the purpose of such determinations merely five cats were used. In connection with these experiments, certain other determinations of the properties of the blood were made, such as the surface tension, the viscosity, the fragility of the red cells,

the cell volume as well as the dry residue of the cells and of the plasma. No certain change in the coagulation time was, however, noted, nor any marked change in the physical properties or composition of the blood in the respects investigated.

In the present author's animal material, findings of emboli and infarcts had not been very frequent. At operation of animals exposed to chloroprene, the author observed, however, that bleeding from the cut surfaces was far less marked than in animals merely narcotized with ethylurethane. In view of this fact and as v. OETTINGEN's investigations had been made merely on a small number of animals, it seemed desirable to take up coagulation determinations for renewed testing on a larger animal material.

For these tests, the author used 40 white rats, weighing from 255 to 330 grams. The coagulation time was estimated (1) before the rats had inhaled chloroprene and (2) afterwards. He used chloroprene which, after stabilization with pyrocatechin, had been kept in a glass bottle without shelter from light. Most previous determinations of the coagulation time in rats seem to have been made on blood taken from the tail. In this way, however, merely small amounts of blood are obtained and it is often difficult to get a spontaneous flow, so that special manipulations have to be adopted, with the risk that fluid from the tissues mingles with the blood, thus affecting the coagulation time. The present author took the blood samples from the carotic artery, after the rats had been narcotized with ethylurethane. The operations were performed with the least possible surgical intervention and with a very small loss of blood.

The effect of different narcotics on the coagulation time has been studied by several investigators both on man and on animals. MENDENHALL (1915) in tests on cats found that chloroform and chloral hydrate did not appreciably change the coagulation time, but that under ether narcosis it was shortened, on an average, by 15 per cent of the normal time. This effect, however, scarcely manifested itself in case the adrenals were removed. In ether narcosis of dogs SEARLES (1939) noted a reduction of the coagulation time by about 9 per cent, and suggests that this might be due to a simultaneously observed increase in the number of thrombocytes.

The present author has not been able to find any statement regard-



ing the direct effect of ethylurethane on the coagulation time, although this substance has long been extensively used as a narcotic in experimental investigations on animals. Thus, FORBES and HOMPE (1921) used cats narcotized with ethylurethane in studies of the effect of carbonmonoxide, illuminating gas and benzole on the coagulation time.

The author, in a small number of cases, has studied the coagulation time in rats before and after narcotizing with ethylurethane of the same concentration and amount as in the coagulation determinations after exposure to chloroprene. The rats received subcutaneous injections of 0.5 ml, per 100 g body-weight, of a 20 per cent ethylurethane solution. In these cases the blood was taken from the tail. In view of the small amount of blood obtainable with this procedure, another method for determination of the coagulation time than in the other tests (referred below) had to be adopted. After an incision in the tail, three drops of blood were collected on a well-cleaned watch-glass. When light rocking of the watch-glass ceased to cause any movement of the blood, it was considered that coagulation had set in. The time from the moment when the blood had been dropped on the watch-glass up to this stage was recorded as the coagulation time. Like all other methods for determination of the coagulation time, this procedure is not exact. Variations in the time required for taking the blood sample may be a source of error. But, in view of the purpose of this investigation, even a method that was not quite accurate seemed to be serviceable. The intention in fact was merely to ascertain whether the ethylurethane narcosis possibly entailed such marked changes in the coagulation time that they must be taken into account in judging the effect of the chloroprene thereon. The result of this investigation, which was made on 10 rats, is shown by the following table.

To judge by the figures, it seems that ethylurethane narcosis, given under the aforesaid conditions, has no effect on the coagulation time.

As pointed out above, the usual methods for the determination of the coagulation time are marred by sources of error. Even if there is some difference in this respect in different methods, the selection of the procedure seems to be of subsidiary importance. On the other hand, it is essential that the technique adopted should

Table VI. Time of coagulation before and after ethylurethane narcosis.

Case No.	Time of coagulation		Difference	Index II/I
	I before narcosis	II after narcosis		
I	2.36	2.25	0.11	95.3
II	3.12	3.30	—0.18	105.8
III	1.67	1.82	—0.15	109.0
IV	2.05	1.53	0.52	74.6
V	2.23	1.95	0.28	87.4
VI	1.86	2.11	—0.25	113.4
VII	2.71	3.02	—0.31	111.4
VIII	3.24	3.28	—0.04	101.2
IX	1.98	1.43	0.55	72.2
X	2.54	2.98	—0.44	117.3
	2.38	2.37	0.09	98.8 ± 5.06

be applied under strictly standardized conditions, in order to obtain comparable values. After testing several different methods, the author adopted the following procedure: —

After inserting a glass cannula into the carotic artery, an equal volume of blood was introduced into the same graduated Elerman tube. It was immediately provided with a rubber stopper, whereupon it was placed in a big centrifugal tube, containing water heated to 19° C. The tubes were then gently rocked while the blood was carefully watched. When it became so viscous that it could scarcely follow the movements of the tube, it was considered that coagulation had set in. The determination of this stage is chiefly a matter of experience, which is a weak point in this and many other methods for determining the coagulation time. The author found, however, that the time within which there was some difficulty in observing this moment did not exceed 10 seconds. His tests showed that this source of error was of no appreciable importance and was outweighed by the simplicity of the method as compared with many others.

When, in this way, an initial value for the coagulation time in animals had been obtained, they were exposed to chloroprene for about 30 minutes. For this purpose, they were placed in a cage with a relatively high, but not exactly fixed, chloroprene concentration, as the object of the experiment was to find out whether exposure to chloroprene, regardless of variations in its

concentration, entailed any change in the coagulation time. After the termination of the exposure the coagulation time was again determined, in the manner above described. The result of these determinations is recorded in the following table: —

Table VII. Time of coagulation before and after exposure to chloroprene.

Case No.	Time of coagulation		Difference	Index II/I
	I before exposure	II after exposure		
1	3.47	3.25	0.22	93.7
2	4.37	1.95	2.42	44.6
3	2.88	2.00	0.88	69.4
4	4.20	2.00	2.20	47.6
5	2.63	2.25	0.38	85.6
6	3.15	1.70	1.45	54.0
7	1.92	0.92	1.00	47.9
8	1.52	1.35	0.17	88.8
9	3.12	0.53	2.59	17.0
10	2.25	2.23	0.12	94.9
11	3.68	1.68	2.00	45.7
12	2.83	1.99	0.84	70.3
13	2.62	1.90	0.72	72.5
14	1.75	1.00	0.75	57.1
15	3.30	2.42	0.88	73.3
16	3.53	4.47	1.06	70.0
17	2.93	2.25	0.68	76.8
18	1.93	1.85	0.08	95.8
19	2.17	1.52	0.65	70.0
20	1.93	1.25	0.68	64.8
21	1.53	1.50	0.03	98.0
22	2.80	1.65	1.15	58.9
23	2.50	1.85	0.65	74.0
24	1.70	1.58	0.12	92.9
25	1.53	1.02	0.51	66.7
26	1.52	1.47	0.05	96.7
27	1.67	1.28	0.39	76.6
28	1.87	1.00	0.87	53.5
29	2.07	2.02	0.05	97.6
30	1.58	1.65	— 0.07	104.4
31	1.70	1.40	0.30	82.4
32	1.53	1.67	— 0.14	109.4
33	2.42	1.37	1.05	56.6
34	2.17	1.40	0.77	64.5
35	2.17	1.95	0.22	89.9
36	2.08	1.57	0.51	75.5
37	1.57	0.97	0.60	61.8
38	2.17	1.92	0.25	88.5
39	1.92	1.50	0.42	78.1
40	1.75	1.52	0.23	86.8
	2.36	1.67	0.69	73.8 ± 1.80

The coagulation time, in all cases but two, was shorter after the exposure to chloroprene than before it. The difference varies considerably as regards the different animals, lying within the limits  $-0.14$  to  $+2.59$  minutes. The mean of the difference was  $0.69$ . The marked deviation may be due to various factors. The coagulation time before the exposure showed a relatively wide range of variation, with a maximum of  $4.37$  minutes and a minimum of  $1.52$ . When the initial value was higher, the difference, as expected, was usually more marked. As the chloroprene concentration was not constant in the different exposures, this too may have conducted to the varying differences. How far this may have been the case will not, however, be discussed in this paper.

It has thus been established that chloroprene has a coagulating effect on the blood. The mechanism, however, has not as yet been ascertained. Here the author merely suggests the possibility that the greater tendency to coagulation, in view of the loss of plasma which has been shown to occur in connection with exposure to chloroprene, may be due to some drying of the blood.

## CHAPTER IX.

### THE EFFECT OF THE CHLOROPRENE ON THE KIDNEYS.

At the postmortem examination of rats that had been exposed to chloroprene, the author, as well as v. OETTINGEN, in a number of cases found degenerative changes of the tubular epithelium in the kidneys. Rather often it was observed to be necrotic. The kidneys moreover showed signs of a general stasis, and sometimes minor hemorrhages were detected in the glomeruli. Renal lesions of such a serious nature have been observed when the animals had been exposed to comparatively high doses. These lesions must evidently have caused a considerable functional disturbance of the kidney, as most of these rats had been affected with anuria, sometimes even during the period of exposure.

In the light of these facts, the author considered it desirable, particularly from a practical point of view, to ascertain how far the renal function was impaired in animals that had been exposed to a smaller amount of chloroprene, and to investigate whether a possible disturbance of function was reversible.

Among various tests of renal function, the author selected a clearance of the ureal nitrogen, mainly for the reason that such a method had been described in detail and carefully tested on rats by FARR and SMADEL (1936). In determining the ureal nitrogen in the blood and urine, the author followed the directions given by OHLSSON (1942) except that it was computed merely in 0.05 ml of blood, instead of 0.1 ml. In order to avoid fluctuations in the clearance value owing to different foods, the rats during the tests received only milk. For the purpose of habituating the rats to a milk diet, it was introduced five days before the beginning of the clearance period. The amount of milk was fixed relatively low, at 30 ml per twenty-four hours, because the rats after the exposure

to chloroprene usually had no craving for any larger amount, and it was considered important that the quantity supplied should be uniform throughout the tests. The milk was received from a glass ampulla provided with a drawn-out neck and fitted for the emission of the milk drop by drop. The whole amount for the twenty-four hours was divided into three portions. During the tests the rats were kept in cages that permitted the collection of the urine separate from faeces or other impurities.

FARR and SMADEL estimate the normal value for urea clearances in rats at  $10.9 \pm 3.1$  ml. As the author, however, used a different diet, it was thought necessary to determine the normal value under these modified conditions. The determinations were made in the course of 28 hours. During this time the urine was collected during two periods of 17 and 11 hours each. At the end of the first period a blood sample was taken. The clearance number was estimated for both periods. The test comprised 11 rats, so that 22 clearance values were obtained. The author found a mean value of  $7.4 \pm 0.4$  ml.

The effect of chloroprene on the renal function was studied by tests on rats who, before the clearance test, had been exposed to a chloroprene concentration of 5 mg per litre of air for 6 hours. The animals were followed up for a fortnight and clearance determinations were made both immediately after the exposure and in the course of the 4th, 6th, 10th and 14th day. Altogether five rats were thus examined. The clearance values found are shown by the table below.

The author found a normal clearance value on the first day, but on the fourth day after the exposure the renal function is considerably disturbed. This disturbance persists, except as regards one of the

Table VIII. Clearance values found in five rats after exposure to chloroprene.

Days	Clearance values found in 5 rats				
	I	II	III	IV	V
0	7.4	7.4	7.9	7.3	7.1
4	1.5	4.1	3.0	0.9	2.7
6	7.2	4.2	5.8	3.2	4.0
10	7.5	5.4	6.4	5.5	5.4
14	7.3	7.9	6.7	10.0	7.4

rats on the 6th and 10th days, but the function nevertheless shows an improvement with higher clearance values throughout. It is seen from the values obtained 14 days after the exposure that the renal function is again satisfactory, with clearance values on a level with the original figures.

The tests have actually shown that, when rats under above mentioned conditions are exposed to chloroprene the renal function is disordered, the clearance value falling to more than 50 per cent of the normal. This fall is most marked in the course of the first few days after the exposure, but the value gradually rises and after 14 days the renal function seems to have been restored to normal.

## CHAPTER X.

### THE EFFECT OF THE CHLOROPRENE ON THE CENTRAL NERVOUS SYSTEM.

When v. OETTINGEN, in his above-mentioned work, states that "Chloroprene affects the central nervous system, as illustrated by the progressive depression observed in all animals used in the different types of experiments," he presumably means that animals exposed to chloroprene show a considerably diminished motor activity. These observations of v. OETTINGEN on changes in the behaviour of the animals in connection with exposure to chloroprene have on the whole been confirmed by the present author in his experiments on animals. They usually sit immobile in their cages, budging only when irritated. They no longer move restlessly searching for food. The present author, however, considered that this change in the animals could not, without further investigation, be attributed to injury of the central nervous system. He therefore thought it desirable to test the effect of chloroprene in this respect with a technique that would permit sufficiently accurate registration of a relatively uncomplicated reaction from the central nervous system. For several reasons, the author found it expedient to study the sensitivity of the nervous system to chloroprene by investigating the narcotic effects of that substance. In analogy with similar experiments with other chlorinated hydrocarbons, it should in fact be possible to obtain a relatively simple reaction in the animals, affording facilities for reliable registrations. In view of the practical purpose of the present work, it was also considered important to throw light on the narcotic effect of chloroprene by experiments on animals. It had in fact happened on some occasions that workers in synthetic rubber factories had been affected with transitory loss of consciousness in connection with intense exposure to chloroprene.



From a practical point of view, it was considered most urgent to try and ascertain whether the narcotic effect varied with different qualities of chlorprene, whereas a gradation of the narcotic effect according to different concentrations was thought to be of minor importance. The author therefore looked about for a method particularly well adapted for the first-mentioned purpose. KNOEFEL and MURRELL'S (1935) technique seemed to meet the case. Its principal features are as follows: — A mouse is put in a glass flask of known volume containing a certain gaseous concentration of the substance the narcotic effect of which is to be tested. If the flask is rotated, the mouse will at first keep its balance, but according as the narcotic effect makes itself felt, it will gradually lose control of its movements. It will slip, roll round, and so forth. LINDGREN (1946) notes a stage where the mouse lies on its back for 30 seconds, and terms the time that has elapsed from the beginning of the experiment up to the end of this stage the "time of induction." By comparing the "time of induction" according to the different substances supplied, relative values for the degree of narcotic effect are obtained.

The present author, adopting a system proposed by AHLMARK (unpublished), noted the times for the reactions "slips for the first time," "dorsal position for the first time," "dorsal position 30 sec." and "lies as still as a parcel." As these stages may be judged differently, it seems necessary to define the precise reactions denoted by these designations. "Slips for the first time" (stage I) as a rule is easily observed if attention is concentrated on the animal's posture. Owing to the slip, the animal momentarily loses its balance and shows a characteristic oblique posture of the body. "Dorsal position for the first time" (stage II) signifies that the animal rolls over on its back. As a rule it immediately recovers its footing. "Dorsal position 30 sec." (stage III), on the other hand, means that the animal lies on its back for the said time. In interpreting this designation, the present author reckoned the time that had elapsed from the beginning of the experiment to the moment when the mouse had lain on its back for 30 seconds. Finally, the designation "lies as still as a parcel" (stage IV) refers to the state in which the animal is unable to get on its feet, despite of many attempts to do so.

For the exposure, the author used a flask having a capacity

of 2.5 litres (height 25 cm, radius 5.6 cm), with a comparatively wide neck and provided with a cork. Through the cork was stuck an 8 cm glass rod at the lower end of which was placed a piece of gauze, on which the desired amount of chloroprene was dropped. The rotation was effected by rolling the flask to and from on a table for a distance of 1 metre and at a speed of about 40 metres per minute. The author first placed the animal in the flask and then dropped chloroprene on the gauze, whereupon the flask was quickly corked and the rolling immediately started.

As, in narcosis experiments with the above-described technique, it is obviously of great importance rapidly to obtain the desired concentration of chloroprene in the flask, the author studied the evaporation rate of the chloroprene as compared with that of ether. For these tests, the author used a sensitive balance and recorded the lapse of time from the moment when a certain equal amount of chloroprene or ether had been dropped on filter paper on one of the scales until the balance had returned to its original position. The conditions with regard to temperature, barometric pressure, etc. were similar to those in the rolling experiments. It was found that chloroprene evaporated very rapidly, and at almost the same rate as ether. This applies particularly to chloroprene that had been kept, after distillation, under nitrogen or carbondioxide in sealed glass ampullae. Under the stated conditions, this quality of chloroprene completely evaporated within 45 seconds. Oxidized chloroprene evaporates at a slightly slower rate than non-oxidized. Polymerized chloroprene likewise evaporates quickly, though not quite completely, which tallies with the well-known fact that the polymeric forms are less volatile than pure chloroprene.

These evaporation tests have thus shown that, under the stated conditions, the intended concentration of pure or oxidized chloroprene is very rapidly obtained, whereas it must be expected that the concentration of the polymeric forms, which, as stated, are less volatile, will not completely correspond to the amount supplied. But, as the author primarily intended to ascertain the differences in the narcotic effect of different qualities of chloroprene, a minor defect in the concentration of the polymeric forms was not considered to be of essential importance.

In accordance with his own experience and that of others, LINDGREN indicates the conditions that should be fulfilled as regards

the animals in narcosis experiments of this kind. He notes that no selection had been made in regard to sex, as both sexes seemed to react much in the same way. As for the weight of the animals, LINDGREN points out that, broadly speaking, there is a direct proportionality between the weight and the "time of induction," as he had in fact shown in a series of tests with ether.

In tests with chloroprene, the present author investigated whether the weight of the animals actually affects the "time of induction." He tested stabilized chloroprene that had been kept for a consider-

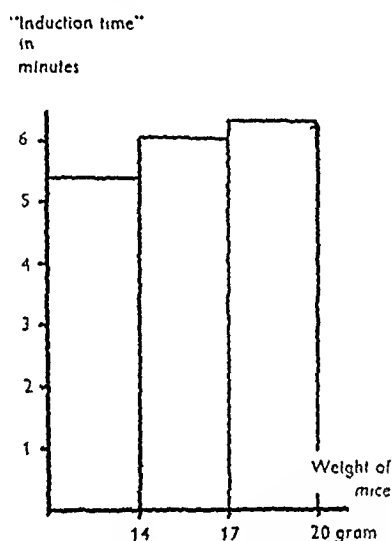


Fig. 10. — Weight and time of induction.

able length of time at room temperature and without being screened from light. The test was made on 50 mice, whose weights varied between 11 and 19 g. As the "time of induction" he reckoned the time that had elapsed from the beginning of the test until the mouse had lain on its back for 30 seconds. He found that the "time of induction" was extended according as the weight of the animals increased; but whether this rise was directly proportional to the increase in weight could not be determined without extending the experimental series. The author's results, however, do not conflict with such a proportionality.

In the narcosis experiments made by the author with different qualities of chloroprene, the weight of the mice averaged  $18.25 \text{ g} \pm 0.27$ , the range being between 15 and 24 g.

The amount of chloroprene supplied in these tests was 0.1 ml, except in the last one, where 0.2 ml was supplied in order to give a satisfying concentration with regard to the eventually less volatility of the polymerized qualities.

The number of animals tested is mentioned under the respective Figure.

As previously pointed out, chloroprene has a great affinity for oxygen and a marked tendency to polymerization. As a rule, therefore, in working with chloroprene, we must expect oxidation and polymerization products, unless special precautionary measures are taken. It seems therefore to be of great interest to try and ascertain how far oxidation or polymerization modified the effect of the chloroprene on the central nervous system. As regards oxidation, it seemed that this matter could be best studied by comparing the narcotic effect of oxidized chloroprene with a chloroprene obtainable, so far as possible, in a chemically pure form. In these experiments, the author made such a comparison with redistilled chloroprene which, after distillation, had been kept under a nitrogen atmosphere in close glass ampullae (this chloroprene is designated here by the letter A).

In narcosis tests with this chloroprene in accordance with the above indicated method, the chloroprene was used immediately after the ampulla had been broken. In this way the test could be carried out within the latency time during which no oxidation had as yet taken place (KLIT). In the author's first experiment (see Fig. 11) the narcotic effect of this chloroprene was compared with the effect of the same chloroprene after it had first been oxidized by causing oxygen to pass through the chloroprene for about 10 minutes (this chloroprene is denoted by the letter A<sub>1</sub>). It is seen from the Figure that the narcotic effect differs considerably in these two qualities. It is already noticeable even after a brief exposure to chloroprene, but becomes more marked according as the narcotic effect increases. With chloroprene A<sub>1</sub>, the narcosis stage III is already reached after 14 minutes, whereas with chloroprene A it is not reached until after the lapse of 26 minutes. As the sole difference in chemical respects that seems to exist between the tested chloroprene qualities is an oxidation, the more powerful narcotic effect of chloroprene A<sub>1</sub> must in all probability be attributed to oxidation products that had been developed. During the brief

space of time required for these tests there is in fact no reason to expect that any polymerization forms had yet been produced.

In Fig. 12 the results from the test with chloroprene (denoted B) obtained by ordinary laboratory distillation of technical chloroprene are illustrated. This chloroprene (B) was used immediately after distillation. Here too there is a distinct difference in the narcotic effect as compared with chloroprene A, though not quite

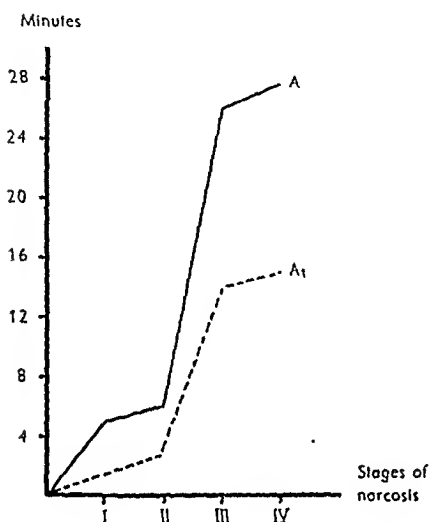


Fig. 11. — Narcosis tests with different qualities of chloroprene.

— Quality A tested on 10 mice  
 - - - - - Quality A<sub>1</sub> tested on 7 mice

The difference ( $12.07 \pm 0.74$ ) between "the induction time" (Stage III) of A and A<sub>1</sub> is statistically significant.

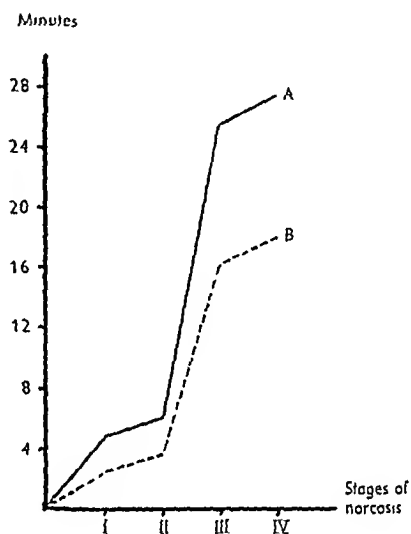


Fig. 12. — Narcosis tests with different qualities of chloroprene.

— Quality A tested on 10 mice  
 - - - - - Quality B tested on 9 mice

The difference ( $9.44 \pm 0.76$ ) between "the induction time" (Stage III) of A and B is statistically significant.

so marked as in the preceding test. In this case too the difference seems to be due to oxidation. It may in fact be presumed that, in view of the marked affinity of chloroprene for oxygen, the conditions in an ordinary laboratory distillation suffice to cause a rapid occurrence of oxidation.

The greatest difference in narcotic effect was observed on comparison with chloroprene which, after distillation, had been stabilized with pyrocatechin and afterwards had been kept for some time in an uncorked glass bottle without screening from light (here

designated B<sub>1</sub>). It is seen from Fig. 13 that the narcosis stage III is already reached with this chloroprene after about 8 minutes. The possibilities of far-reaching oxidation had evidently been very favourable with this chloroprene, and it is also probable that the powerful narcotic effect had been mainly due to oxidation products. It is, however, conceivable that a certain amount of  $\beta$ -polymers may also have been formed and conducted to the said narcotic

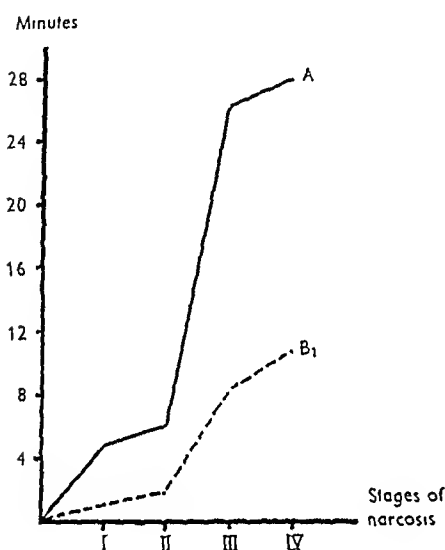


Fig. 13. — Narcosis tests with different qualities of chloroprene.

—— Quality A tested on 10 mice  
 ----- Quality B<sub>1</sub> tested on 15 mice

The difference ( $17.57 \pm 0.74$ ) between "the induction time" (Stage III) of A and B<sub>1</sub> is statistically significant.

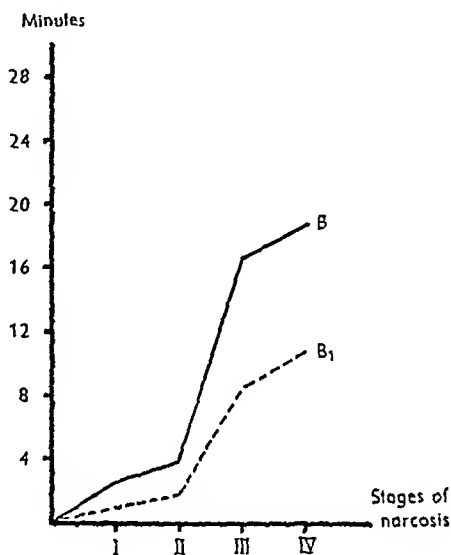


Fig. 14. — Narcosis tests with different qualities of chloroprene.

—— Quality B tested on 9 mice  
 ----- Quality B<sub>1</sub> tested on 15 mice

The difference ( $8.13 \pm 0.84$ ) between "the induction time" (Stage III) of B and B<sub>1</sub> is statistically significant.

effect. As the author shows below, the narcotic effect increases according as the polymerization proceeds.

In Fig. 14 the author has compared the narcosis tests with laboratory-distilled chloroprene (B) and pyrocatechin-stabilized chloroprene (B<sub>1</sub>). This comparison is of special interest, as these two qualities represent chloroprene that had often been used in laboratory experiments, but which also occurs in the production of synthetic rubber on a manufacturing scale. The narcotic effect is relatively high in both cases, but distinctly higher for the chloro-

prene that had been exposed to air and light for a considerable length of time.

The author considered it also of interest to ascertain whether the narcotic effect was influenced by the degree of polymerization. He accordingly allowed newly distilled chloroprene to stand for one day, or for two days, without a stabilizer. In comparing these qualities and freshly distilled chloroprene, Fig. 15, one finds a certain difference, though on a minor scale. As previously

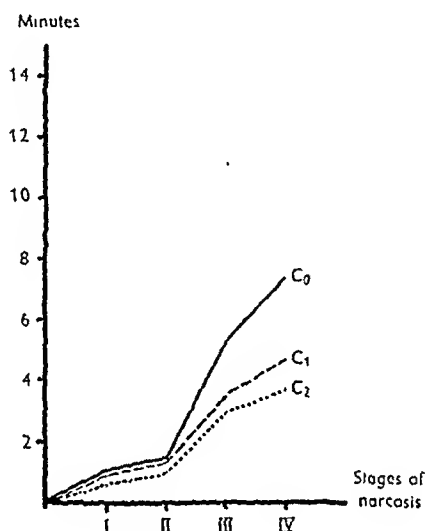


Fig. 15. — Narcosis tests with different qualities of chloroprene.  
 — C<sub>0</sub> — non-polymerized chloroprene tested on 15 mice  
 - - - C<sub>1</sub> — chloroprene, polymerized for 1 day tested on 5 mice  
 . . . C<sub>2</sub> — chloroprene, polymerized for 2 days tested on 5 mice  
 The difference ( $2.26 \pm 0.42$ ) between "the induction time" (Stage III) of C<sub>0</sub> and C<sub>2</sub> is statistically significant.

noted, reservation must be made for the possibility that the polymeric forms may not have completely corresponded to the amount supplied, as the polymers are less volatile than pure chloroprene. The obtained effect is likely to be greater if the evaporation had been total. Moreover, it is conceivable that the change in the narcotic effect, at any rate in part, may have been due to oxidation products, the formation of which cannot be avoided in tests like these.

## CHAPTER XI.

### POSTMORTEM FINDINGS.

In the following an account will be given of microscopical findings mainly from organs which it was of special interest and importance to examine in connection with the present author's experimental investigations on animals. Firstly, as regards the lungs, a comparison with control animals was as a rule made, as it is by no means unusual that the lungs of laboratory animals "normally" show several pathological findings.

In the acute tests hyperemia with edema was the commonest finding also in the present author's material. The edema was most marked in the inhalation tests, but occurred also after injection of chloroprene. In some lungs there was not only an efflux of fluid to the alveoli, but also an interstitial edema. In such cases one could rather often observe thin-walled, greatly distended alveoli and, in several preparations, a distinct emphysema occurring in patches, especially in the marginal parts of the lungs. This emphysema usually developed rapidly in the course of one or two hours, and may be regarded as a so-called compensatory emphysema. A similar picture is well-known from phosgene poisoning (LAQUEUR and MAGNUS, 1921), though in such cases the edema in the lungs as a rule is more marked than after exposure to chloroprene.

In the experiments in which the present author tested oxidized chloroprene, the pulmonary damage, with the same amounts of chloroprene, was more marked than when freshly distilled chloroprene was employed. In long-continued inhalation tests on rats



exposed to a concentration of 0.2 mg per litre of air for 8 hours a day in the course of five months (see Ch. V B), the pulmonary changes were inconsiderable. In some cases a purulent bronchitis occurred with round-cell foci round the bronchi. But, as this symptom was found also in one of the control animals, and as it is not a rare phenomenon in laboratory animals even if they have not been used for experimental purposes, these findings cannot be attributed to the effect of chloroprene. In the experimental series where the animals were exposed to a chloroprene concentration of 1.2 mg per litre of air during the same period as above stated, a slight hyperemia usually occurred in the lungs, and in some cases minor hemorrhages in the parenchyma. Symptoms of a moderate pulmonary edema were observed in some of the animals, but no emphysema.

Pathological changes in the heart have been very sparse in the author's material. In the acute tests no cardiac lesions could as a rule be observed macroscopically or under the microscope. After inhalation of chloroprene (1.2 mg per litre of air) for some length of time, slight degenerative changes in the heart muscles, with sarcolysis in a few cases, occurred in some of the animals.

The most marked changes in connection with exposure to chloroprene were found in the liver. After a short exposure with relatively low concentrations of chloroprene, they consisted of a more or less marked, mainly capillary, hyperemia. At higher chloroprene concentrations degenerative changes occurred, with decomposition of the liver-cells, especially periportally. In some preparations necrotic foci were observed here and there. As a rule fatty changes occurred merely to a slight extent. In the animals that had survived the tests for some length of time, there were signs of reactive processes, with granulation tissue.

The renal lesions were mostly confined to the tubuli contorti, with degenerative changes of the tubular epithelium and here and there slight fatty changes. These changes, however, did not appear until after a relatively intense exposure to chloroprene. When the chloroprene concentrations amounted to 5 mg per litre of air or more for 6 hours, the glomeruli were also affected, with exudate in the capsular spaces.

In some brain preparations from rabbits that had been caused to inhale chloroprene, a moderate hyperemia was observed throughout

in the cerebral and meningeal blood vessels as well as a perivascular edema; but no hemorrhages could be seen.

As previously mentioned, a case of mortality due to acute chloroprene poisoning occurred among the workmen in connection with the production of synthetic rubber in Sweden. This happened in the cleaning of a "latex" container (polymerization vessel) which was to be freed from accumulated rubber waste. The workman in question went down into this container without observing the prescribed precautions, such as blowing air through it. The vessel therefore came to contain residual gas consisting mainly of chloroprene and which, owing to its weight, had been concentrated in the lower part of the vessel. When the man had been about 20 to 30 seconds on the bottom of the container, he was observed to stagger and collapse. He was extricated from the container after about three or four minutes, but then showed no sign of life, and all attempts at resuscitation were in vain.

At the postmortem<sup>1</sup> the chief changes found were in the lungs and air passages. The lung tissue everywhere contained an abundant amount of a rather thin, blood-coloured fluid. In the anterior parts of the lungs this fluid contained air. In the larynx, trachea and bronchi the same kind of blood-tinger fluid was found, but here mingled with much froth. The microscopic examination of the lungs showed marked hyperemia of the blood vessels and a copious amount of thin fluid everywhere in the lung tissue.

The course of events in this case, the postmortem findings as well as observations from experiments on animals make it possible to judge the probable cause of the death. The workman had very soon (after about 30 seconds) lost consciousness, so that he was unable to keep his balance. This effect was doubtless due to the action of the chloroprene on the central nervous system. The experiments on animals had shown that chloroprene has a strong narcotic effect (see Ch. X) and the author has pointed out that acute cases of poisoning with transient loss of consciousness had occurred in the factory premises before the hygienic conditions had as yet been improved. The rapid sequel indicates that the workman had been exposed to a very high chloroprene concentration which probably had soon led to a central respiratory arrest.

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<sup>1</sup> The postmortem examination was made by Docent H. SJÖVALL, M.D., the State Institute for Medical Jurisprudence, Stockholm.

Parallel with this effect, the gas had had a direct action on the respiratory passages, which had resulted in a pulmonary edema. It is not improbable that this edema to some extent had been also caused by a stasis in the pulmonary circulation by a failure of the heart action, in analogy with the observations made by the author in experiments on animals.

## CHAPTER XII.

### DISCUSSION.

The experimental investigations on animals have, in several cases, shown that oxidation as well as polymerization changes the pharmacologic properties of chloroprene. The author has, for example, found that the mortality in rats is considerably greater on the subcutaneous injection of oxidized, as compared with non-oxidized, chloroprene and, in studying the effect of chloroprene on the central nervous system that the narcotic effect increases on oxidation and polymerization. The "supermortality" observed on the injection of oxidized, as compared with non-oxidized, chloroprene should probably be attributed to a cardiopulmonal insufficiency in the animals to whom the first-mentioned quality of chloroprene had been administered. The lungs of those animals in fact showed a continuous increase in weight, with intensification of the edema and stasis, according as chloroprene was supplied in increasing amounts.

In view of the author's investigations into the effect of chloroprene on the heart, it may be presumed that the pulmonary changes are to some extent attributable to a failing heart action. In all probability, the oxidized chloroprene with its acid products also has a directly injurious effect on the lungs, with resulting edema. This is indicated by the fact that the pulmonary edema was particularly marked on the inhalation of oxidized chloroprene. The subcutaneous method of administration does not rule out the possibility of a directly injurious effect on the lungs, as such an effect may occur after chloroprene has been supplied to them via the blood. A similar mechanism has been described by DEICHMANN *et al.* (1944) on the injection of kerosene into rats.

In recording the blood pressure at the carotic artery in rabbits and cats in connection with inhalation of chloroprene, the present

author has verified v. OETTINGEN's results from similar experiments. The present author considers the fall of the blood pressure to be a manifestation of a depressant effect on the heart. He in fact observed a distinct effect of this nature in experiments with chloroprene on isolated rabbit's and frog's heart. It seems hard to understand that v. OETTINGEN did not observe any such effect in analogous experiments on frog's heart in accordance with Straub's technique. Even if different qualities of chloroprene had been used in v. OETTINGEN's and the present author's experiments, this scarcely seems to be a convincing explanation, as the author could not observe any ascertained difference in the effect of different qualities of chloroprene on the blood pressure in experiments on rabbits in vivo. In default of a direct effect of chloroprene on isolated frog's heart, v. OETTINGEN supposed that the observed fall of the blood pressure from the in vivo tests was attributable to a, presumably active, dilatation of the abdominal blood vessels.

It seems to the present author that there are no cogent reasons for such an assumption. The frequently occurring hyperemia in the abdominal organs after exposure to chloroprene should, instead, be regarded as a stasis hyperemia due to failing heart action. In perfusion experiments on frogs in accordance with Trendelenburg's technique, v. OETTINGEN found a vasoconstrictory effect of chloroprene on peripheral vessels, but he does not report any tests that would have shown a dilatatory effect of chloroprene on the abdominal vessels.

As previously mentioned, the increase in the hematocrit value of the blood may also be referred to disturbance of the circulation and should be mainly attributed to a loss of plasma in connection with pulmonary edema due to stasis in the pulmonary circulation. It seems moreover by no means improbable that the viscosity of the blood may be increased by the loss of plasma, thus intensifying its coagulative tendency. The mechanism would thus be analogous with that in phosgene poisoning, where the coagulation time is reduced owing to drying of the blood. True that v. OETTINGEN did not find any increase in viscosity in his experiments on cats, but, as no particulars are given about the quality of chloroprene used, no decisive importance can be attached to this test.

The anemia observed in connection with long-continued inhalation tests on rats should probably be attributed to a toxic injury of

the blood-forming organs and can scarcely be of alimentary character, even if this might be indicated by the simultaneous loss of weight. In fact, setting aside the first few days after the experiments had been started, the ingestion of food by the exposed animals was, broadly speaking, quite equal to that of the controls. — The degenerative changes in parenchymatous organs, especially liver and kidneys, may also be regarded as an indication of injurious toxic effects on the cells.

Finally, as regards the reduced oxygen content of the blood after exposure to chloroprene, the author has shown by his experiments that it is in all probability due to a diminished oxygen capacity in the blood. Though the author considered it most probable that this diminution in the oxygen capacity was due to a formation of methemoglobin, he has not yet been able to adduce any convincing evidence in support of this view.

## Examination of the Workers in the Synthetic Rubber Industry.

### CHAPTER XIII.

#### INFORMATION ABOUT THE EMPLOYEES.

In Chapter II it has been pointed out that, on examination of the men employed in the synthetic rubber industry, it was found that only the workers in certain departments showed signs of ill-health owing to their work. The said groups of workers have been subjected to thorough examination, whereas the other employees have been more cursorily examined. With some few exceptions, all the examinations were made at the place where the work was carried on and thus in close connection with the exposure. This was considered important, as some of the symptoms shown by the workers vanished or abated rather soon after the termination of the day's work.

Repeated medical examinations of the workers were made by the author in the course of the years 1944—1947. As most of the workers in this industry had been permanently employed during these years, the examinations involved continuous control of the same persons. It may be noted that only male workers were employed in the factories. Their age distribution is shown by the following table.

Merely a few of the workmen in the rubber industry had previously been employed in any other chemical industry. Most of them had been employed in sawmills or else in lumbering or agriculture. As a rule, heavy manual labour does not occur in the synthetic rubber industry, the work consisting largely in attendance

to apparatus. The work is carried on in three shifts, with weekly periods. The changes of shift are timed at 6, 14 and 22 o'clock.

The rubber factory where the most thorough medical examinations were made is situated in a sparsely populated district, and most of the men lived a long distance, 20 kilometres or more, away from the factory. While the manufacture was in an experimental stage merely some thirty workers were employed, whereas at a later date, when the industry had been extended, they numbered about two hundred. All the men who might conceivably have been exposed to any noxious substances from this manufacture were

Table IX. Age distribution.

Age	Number
15—19.....	4
20—24.....	22
25—29.....	34
30—34.....	21
35—39.....	30
40—44.....	12
45—49.....	14
50—54.....	18
55—59.....	15
60—64.....	3

subjected to medical examination on at least one occasion. The more continuous and thorough examinations were made in the case of workers who showed symptoms of disease. As already mentioned, they were found mainly in the distillation and polymerization departments.

In the medical examination of the workers, the author endeavoured to obtain the most detailed possible anamnesis — especially as regards their social situation. The state of health and performance of a shift worker obviously must be closely dependent on his opportunities for recovery during his free time. Most of the workers in this industry had had difficulties with the housing problem and had rarely been able to procure a dwelling where they could obtain satisfactory rest or sleep in the daytime after a night shift.

The way in which the workers spent their leisure time, especially as regards athletic sports and physical work, was followed with attention, as the author considered that such initiatives were good



<i>Name:</i> <i>Adress:</i>	Born on the    /    I ..... Occupation:
Previous employment: Employed in the synthetic rubber industry since: Principal work in the factory: Exposure to Chloroprene:	
Hereditary conditions:	Previous diseases:
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <i>Subjective symptoms:</i>            Fatigue (impaired state of health):            Pressure and pains over the chest:            Palpitation of the heart:            Headache:            Giddiness:            Irritability:            Dermatitis:            Loss of hair:            Other complaints:         </div> <div style="width: 45%;"> <i>Observed first time:</i> </div> </div>	
<i>Status:</i> General condition: Throat and oral cavity: Heart: a) percussive heart limits: b) sounds: c) rythm: Blood pressure: Pulse: Pulm: Abdomen: Skin: Reflexes: a) pupillary reflex: b) patellary reflex: c) Babinsky: Romberg:	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <i>Laboratory tests:</i>            SR:            HB:            Red cells:            White cells:            Differential count:         </div> <div style="width: 45%;">           Heller:            Almén:            Urine sediment:            Schlesinger:         </div> </div>	

Fig. 16. The examination was made in accordance with this schedule, but with some deviations where necessary.

indications of their physical and mental health and corresponded well with the hygienic conditions at the establishment where they worked. Soon after this industry had started most of the workers were incapable of any work or other effort during their free time and had to devote it entirely to rest. But, according as the medical examinations proceeded and the managers or foremen could be given suggestions or instructions for improvement of the hygienic conditions, the leisure-time occupations were resumed and before long they were being carried on to quite the same extent as by workers in other industries.

In studying the workmen's anamnesis, it was found, that merely a few of them had had any serious diseases. Those cases where it was conceivable that an impaired condition of health might possibly be due to a disease which had formerly been passed through were not included in the author's material. A similar remark applies in regard to any temporary ailment that might be expected to impair the worker's capacity.

As regards the symptoms (found in examinations according to the schedule in page 86) of disease among the workers and their distribution according to the different departments, the reader is referred in essentials to the statements made in Chapter II. The differences or special features in this respect found in the different factories will be mentioned further on in connection with a report on the investigations in those establishments.

## CHAPTER XIV.

### AT THE PILOT PLANT.

#### A. *Distribution of symptoms.*

The author's first medical examination of workers in the rubber industry was made at the beginning of 1944 at a pilot plant, which had then been in operation merely for a comparatively short period. This factory was housed in old-fashioned, cramped premises with defective hygienic conditions, and lacked special safety regulations for the workers. As the different departments were not properly separated, and as many of the workers circulated from one department to another, it was not possible to form any distinct idea as to how the symptoms were distributed according to the different departments. In the table below the distribution of the symptoms is estimated on the basis of the whole number of workers (thirty) in this factory, without reference to departments.

Thus, despite the circulation of the workers in this factory between different departments, the incidence of disease symptoms

**Table X. Distribution of symptoms among the workers at the pilot plant.**

S y m p t o m s	The pilot plant 30 workers	
Fatigue (impairment of health) . . . . .	12	40 %
Pressure and pains over the chest . . . .	9	30 %
Palpitation of the heart . . . . .	3	10 %
Giddines . . . . .	3	10 %
Irritability . . . . .	5 ca.	17 %
Dermatitis . . . . .	6	20 %
Loss of hair . . . . .	3	10 %

among them was high. As the author had pointed out, this was due to hygienic defects of different kinds in the factory. The distribution of the different symptoms in this factory turned out to be very similar to that which the author afterwards found at other factories which were far more up-to-date.

The somatic examination of the workers at the factory in accordance with the above schedule yielded very few objective findings. In most cases, moreover, they could scarcely be supposed to have any connection with the work at the factory and, generally speaking, were already known to the men before they were employed in the synthetic rubber industry. There occurred, for example, a case of Addison's disease, as also one of asthmatic bronchitis, and in two of the men a blowing cardiac murmur was heard. These men had previously been examined at a medical clinic and it was considered that they had no organic heart disease.

The result of the investigation as a whole indicated mainly a very satisfactory state of health among the workers, which badly corresponded with the subjective symptoms that occurred among them.

## B. Blood examinations.

As regards the laboratory tests, only the hemoglobin and red cell figures will be discussed here, as they were the only findings that showed pathological values. These blood values were determined by the author on four different occasions in the course of the years 1944—1945. For the Hb-determination he used a standardized Sicca hemometer ( $100\% \text{ Hb} = 19.0$  volume per cent oxygen) and made the sampling and readings in accordance with the instructions given for the use of this apparatus. The blood-cell count was made with the aid of a Bürker chamber in the usual way. — In the table below the mean values for hemoglobin and red cells from the thirty workers at the different examinations are recorded: —

Table XI. Hemoglobin and Erythrocytes (15 workers).

Occasion of examination	Hemoglobin	Erythrocytes
Januari 1944.....	$79.9 \pm 1.06$	$4.387 \pm 0.215$
May 1944.....	$86.3 \pm 1.33$	$5.109 \pm 0.103$
November 1944.....	$103.6 \pm 1.62$	$5.176 \pm 0.053$
Februari 1945.....	$108.9 \pm 1.21$	$5.106 \pm 0.086$

It will be seen from these figures that the hemoglobin and, in a minor degree, the red cell counts were lower at the three first examinations than later. The sample last taken shows values that correspond well with those found by ENGHORF in 1937 among the population from the place where the factory was situated. ENGHORF estimates the number of red cells for healthy men between the ages of 18 and 29 years at  $5.4 \pm 0.06$  million, and the oxygen capacity of the blood for the same age-group at  $21.5 \pm 0.28$  volume per cent.

If the latter value is converted into a Sicca value in accordance with the apparatus used by the author, we find a hemoglobin percentage of 113.1. According to this comparison, the workers at the medical examinations in January and May 1944 appear to have had a distinctly hypochrome anemia, which, however, showed an improvement in the course of the year, so that at the beginning of 1945 the blood values were, broadly speaking, normal.

Even if these workers had presumably been exposed to several of the substances formed incidentally during the manufacturing process, there is nevertheless good reason to suppose that the anemia had been caused by chloroprene. The author has in fact shown (see Ch. VIII A) that a hypochrome anemia developed in rats exposed for some length of time to air containing chloroprene at a concentration of 1.2 mg per litre of air. The author, unfortunately, has no information regarding the chloroprene concentration in the premises of this factory, as no suitable method for the determination of chloroprene in air was known to him at this time of the investigation. But, in view of the fact that the chloroprene concentration in the newly built factories with far better resources for ventilation occasionally amounted to 1.65 mg per litre of air (the distillation department), it seems highly probable that the workers at the pilot plant had been exposed to such high chloroprene concentrations as to permit the development of anemia. The progressive improvement in the workers' blood status observed at examinations at the end of 1944 and beginning of 1945 may be attributed to the sanitary improvements made in the course of 1944. The chloroprene concentration necessary to cause the development of anemia seems to be about 1.2 mg per litre of air. In the author's experiments on animals no anemia occurred with a concentration of 0.2 mg per litre of air, nor did he observe any

anemia among the workers at the other factories, where, at any rate to begin with, the chloroprene concentration in certain departments often ranged between 0.2 and 0.8 mg per litre of air.

The anemia observed among the workers, however, could scarcely be the real cause of their fatigue, nor did it serve to explain their other symptoms. It was in fact found at later examinations that the symptoms persisted even after the anemia had been abolished and that similar symptoms occurred also in the two other factories where the workers had no anemia.

### C. Schneider Index.

In view of the effect of chloroprene on the heart, observed in connection with the animal experiments, one of which was a decrease of the arterial pressure, it seemed appropriate to devote special attention to the state of the organs of circulation among the workers. As the symptoms from the thorax, with pain and pressure localized in the region under the sternum were specially marked during exertion, it was thought desirable to make these investigations in connection with a working load.

For practical reasons, it was found necessary in the first place to adopt a procedure that could be carried out on the premises without interfering with the work of the employees at the factory. The author decided therefore, as a preliminary survey to record the Schneider index. This test has a limited value and has been recommended by SCHNEIDER (1920) himself chiefly as a supplement to a general clinical examination. The possibility of judging cardiovascular states of insufficiency with the aid of this test has been discussed e. g. by FEIL, PETTI and PARK (1943). It has been pointed out by SCOTT, BAZETT and MACKIE (1940) that repeated records of the Schneider index for the same individual for a certain period of time can give valuable information regarding changes in his capacity for work. Subject to certain reservations in regard to the reliability of the test, it seemed therefore justifiable to adopt the method as a supplement to other examinations. Merely four workers were tested for some length of time in accordance with this method. The author in fact followed only those workers whose Schneider index had been recorded when they took up their posts, or before any symptoms of ill-health had begun to manifest them-

selves. The examinations were repeatedly resumed in the course of six months, during which time all these workers began to show marked symptoms of fatigue and thoracic pain. The tests were made in each case under as similar conditions as possible.

Before the testing had started, the workers had been allowed to rest for about half-an-hour. For the procedure in other respects, readers are referred to SCHNEIDER's directions, which the author completely followed. — In the subjoined table, the mean values of the Schneider index for the four workers are computed for three periods. The first period comprises the time pending the manifestation of the symptoms, the second period the time in the course of which the symptoms developed to their full intensity, and the third period the time afterwards.

Table XII. Schneider Index.  
(Mean values of 8 determinations.)

Case	I	II	III
A	12.4 $\pm$ 0.4	12.0 $\pm$ 0.4	12.4 $\pm$ 0.3
B	14.5 $\pm$ 0.3	15.0 $\pm$ 0.3	14.3 $\pm$ 0.4
C	15.4 $\pm$ 0.3	15.4 $\pm$ 0.3	15.8 $\pm$ 0.3
D	13.0 $\pm$ 0.3	13.3 $\pm$ 0.4	12.9 $\pm$ 0.2

The figures thus obtained show that the mean values for the index during the different periods vary very slightly for the same worker, and that there is no significant statistical difference. Thus, it did not seem possible by this procedure to obtain any objective view as to the effect of the work on the employees.

Two of the workers who had been tested in accordance with the above procedure afterwards were sent for examination at the Physiological Institute in Uppsala. With the aid of ENGHOF's baro-spirograph (1939), the respiratory minute-volume during dosed work on the cycle ergometer was determined. When compared with the normal values obtained by ENGHOF at examination of healthy persons under similar experimental conditions, no deviations from the normal could be observed.

## CHAPTER XV.

### AT THE FACTORIES.

#### A. Distribution of symptoms.

When the production of synthetic rubber had got beyond the experimental stage, operations were started on a manufacturing scale at a newly built factory, which employed about 80 workers. This factory was housed in modern premises, where the experience, in various respects, from the pilot plant had been turned to account. In particular, steps had been taken for improvement of the hygienic conditions. In view of the precautionary measures adopted, it might have been expected that the workers in this factory would have been less affected than those at the pilot plant.

This, however, was by no means the case when operations at this factory were first started. Only after the lapse of some time, when the sanitary conditions had been brought into order, did the symptoms begin to recede. Thanks to the strict organization of the different processes of production in different premises, and as the workers were stationed at the same places throughout, it was easy to locate the places in the factory where they were exposed to risks. As previously mentioned, it was only the workers in the distillation and polymerization departments that showed symptoms of ill-health, whereas the state of health in the other departments was very satisfactory. The symptoms there were of the same character as among the workers at the pilot plant and quite as marked. The incidence of the symptoms is shown by the following table in next page.

Except for the loss of hair, which was most marked in the polymerization department, it was especially the workers in the distillation department that showed symptoms of disease. This distribution



Table XIII. Distribution of symptoms among the workers at one of the factories.

S y m p t o m s	Distillation department (21 workers)	Polymerization department (12 workers)
Fatigue (imperment of health) .....	19 ca. 90 %	1 ca. 10 %
Pressure and pains over the chest ....	19 ca. 90 %	1 ca. 10 %
Palpitation of the hearth .....	5 ca. 25 %	1 ca. 10 %
Giddiness .....	3 ca. 15 %	1 ca. 10 %
Irritability .....	6 ca. 30 %	0 0 %
Dermatitis .....	5 ca. 25 %	4 ca. 30 %
Loss of hair .....	0 0 %	11 ca. 90 %

of the symptoms is of special interest because the fact that the oxidized chloroprene, in accordance with the author's experiments on animals, in several respects was pharmacologically more active than the polymeric forms, except, however, for the loss of hair.

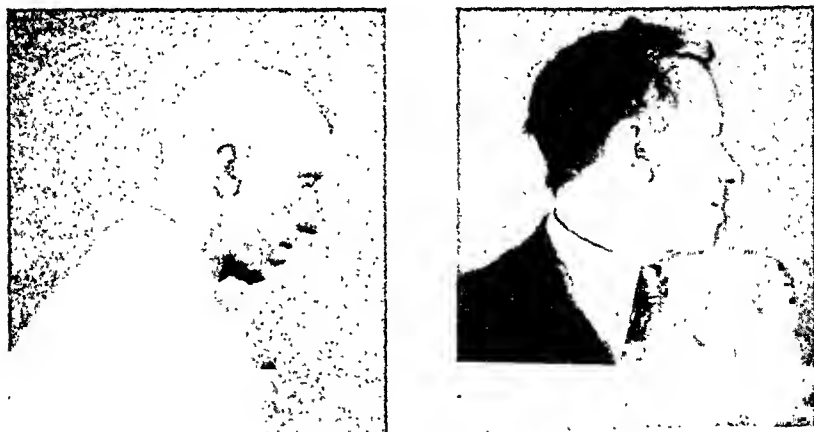


Fig. 17. — Left: Loss of hair after about two months' work at the polymerization department.  
Right: The same worker about seven months after the termination of his work at that department.

It should be pointed out that the above table represents the situation at a particular examination. If consideration is paid to the state of health for a considerable length of time, the percentage figures, especially for "fatigue" and "pressure over the chest" in the distillation department and the "loss of hair" in the polymeriza-

tion department would be still higher, lying very close to a hundred per cent.

The state of health at the second of the synthetic rubber factories will be dealt with quite summarily, as, in size and lay-out, it closely resembled the one above described. The distribution of disease symptoms among the workers was likewise similar, but their incidence was less, which seems to be due to the fact that this factory, which started its manufacturing operations later than the other, had been provided from the outset with effective safety arrangements. It was only in regard to the loss of hair that this factory shows the highest incidence. As previously mentioned, it was only the workers in the so-called mass polymerization department that were affected, almost without exception, by loss of hair. For technical reasons, it was very difficult effectively to protect the workers against fumes from chloroprene polymers, issuing from the apparatus in this department. Not until this process had been superseded by another, more suitable from a sanitary and technical point of view, did the falling-off of the hair almost entirely cease.

The examinations of the workers in conformity with the above outline yielded no findings of importance in this connection. But, in view of the subjective symptoms and the observations made in the experiments on animals, it was considered desirable to make certain special investigations, an account of which will be given below.

## **B. Functional tests on the kidneys and the liver.**

In view of the renal damage observed in the experiments on animals, with distinct impairment of the renal function even after a relatively short exposure to chloroprene, a renal-function test in accordance with REIBERG's (1926) creatinin clearance was made as regards ten of the workers most affected. This test is well suited in field work, as it is easily performed and does not occupy the tested subject for any length of time. The test is mainly a gauge of the glomeruli filtration, but to some extent also of the function of the tubuli, as creatinin is partly excreted through the tubular epithelium. (SMITH, 1937.) The normal values for this method are stated to be 100—180 ml per minute. For practical reasons, it was not possible to carry out these tests during strict confinement to bed, but the workers were afforded opportunity for rest in a recumbent position at the establishment while the tests were proceeding. Under such conditions there seems to have been but little risk of any marked changes in the clearance value because of in-

sufficient rest. The tests were made in the morning on an empty stomach. The samplings and analyses were made in accordance with REHBERG's directions. The figures for the values found are recorded below: --

Table XIV. Values of Creatinin Clearance.

Case	Filtration in ml/min.
O. H. ....	127
A. M. ....	162
O. G. ....	126
K. E. ....	124
O. H. ....	148
E. S. ....	119
O. A. ....	176
K. K. ....	138
I. O. ....	110
E. Q. ....	123

The above tabulation shows that the clearance values for all the tested subjects lay within normal limits.

In experimental investigations on animals, v. OETTINGEN in some cases found signs of liver insufficiency, and the present author also in his experiments on animals on a few occasions found a positive Schlesinger's reaction after exposure to chloroprene. Regular control of workers in the chloroprene industry are recommended by v. OETTINGEN, in view of the possible occurrence of bile-pigments in the urine. The present author has in no case found a positive Schlesinger reaction during the time when the workers in the synthetic rubber industry were being subjected to health control. Nor, when liver-function tests were made did he observe any objective signs of liver damage. Functional tests were made as regards 20 workers, hippuric acid and thymol, respectively, being used for the test in 10 of these cases. In the first-mentioned test 6 g of sodiumbenzoate was supplied per os. The normal excretion of hippuric acid in the course of four hours after administration is stated to be 3 g, with individual variations between 2.55 and 3.30 g. For all the men tested the values lay within those limits. The mean value for the 10 workers tested with hippuric acid was 3.12 g (range 2.70—3.18 g). Clinical experience has shown that the excretion of hippuric acid is diminished in cases of hepatitis,

cirrhosis and often in liver metastases. The test seems to give a reaction even where the damage to the parenchyma is rather moderate. It is not so sensitive, however, as the thymol test, which in this respect seems to be comparable with the Hanger test (BRANTE, 1946).

The 10 workers that had been tested with thymol had worked in the distillation and polymerization departments for about two years. All of them had at times shown very marked symptoms of fatigue as well as pain and pressure over the thorax. In none of these cases were values over 4 E. obtained. The normal values for the thymol test are stated to lie under 6 E.

### C. Basal Metabolism.

According to a verbal communication from the Du Pont Co., Wilmington, Delaware, U.S., a strikingly large number of the chloroprene workers there are said to have had a lowered basal metabolism.<sup>1</sup> According to one of the statistical reports from that company, merely two out of 19 workers had shown normal values, viz.  $100\% \pm 10$ . As a rule, however, the falling tendency was relatively moderate.

The present author determined the metabolism in 10 workers chiefly employed in the distillation department. This investigation was made towards the termination of the observation period (1944—1947), when the sanitary conditions in the factory were satisfactory and the men, generally speaking, showed very slight symptoms. For practical reasons, this investigation had to be conducted on the premises. It was made in the morning after a fast of about 12 hours. Unavoidably, some of the workers examined had been exposed before the test to some exertion, having had to cycle on their way to the factory. The determination was made in the usual way with the aid of a Krogh spirometer.

The result of the investigation is recorded in the table in next page.

The last value (No. 10) in this table may be eliminated from discussion here, as it was found that the worker in question was suffering from diabetes mellitus, which may explain the high B.M.B. The high figures for Nos. 8 and 9 may be accounted for by the fact

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<sup>1</sup> Personal communication from Dr. George H. Gehrman.

Table XV. Values of Basal Metabolism.

Case No.	B.M.B. %	Means of travelling to the workplace
1	- 12	By train
2	+ 6	»
3	+ 8	»
4	+ 9	»
5	+ 10	»
6	+ 12	»
7	+ 13	»
8	+ 16	By cycle 5 miles
9	+ 22	By cycle 3 miles
10	+ 76	By train

that they had cycled to the factory. The workers representing the values 2—7 all show plus values, which, however, lie within, or very near, the upper limit for the normal. Only one of the workers (No. 1) had a lowered basal metabolism, with a value of - 12. This figure thus lies slightly below the lower limit of the normal value, - 10, but, when compared with the other values and in view of the fact that the examination was made under ambulatory conditions, this value probably represents a lower B.M.B. than the figure indicates. It is noteworthy that this worker was the only one of those examined in this respect who still complained of symptoms such as bad general condition, dyspnea, palpitations as well as pain and pressure over the chest.

These investigations have not given an exhaustive reply to the question as to how far the metabolism is affected by chloroprene: for this purpose, further investigation seems to be necessary. As the workers in the sequel were merely quite slightly exposed to chloroprene in the factory, it did not seem possible to obtain a larger material from there. In connection with proceeding experimental investigations on animals in regard to the intermediate metabolism of chloroprene in the organism, the author intends to study this problem also.

#### D. Mass miniature radiographic examinations of the lungs.

According to the above-mentioned American source, the x-ray examination of the lungs showed that a comparatively large number of workers in the chloroprene industry had symptoms of a proceed-

ing or former pleuritis. To judge by the physical routine examination, it seemed scarcely probable that this was the case at the factories in Sweden. But, as it did not seem possible to give a reliable answer to this question without an x-ray examination, a mass miniature radiography of all the workers at one of the factories, comprising 80 employees, was carried out. It was found that 67 of them, roentgenologically, had normal lungs. In 8 cases unilateral or bilateral sinus obliteration was found. In two of the workmen minor parenchymatous changes, which have been subjected to further examination, were detected.

As a basis of comparison, mass miniature radiography of about 400 workers employed in other chemical industries at the same place was simultaneously carried out. The production at these factories consisted mainly of ammonia and nitric acid. When the records were studied, sinus obliteration was found in a somewhat larger number of workers than in the rubber industry. Otherwise the x-ray findings were few and without significance in this connection.

How far the pulmonary symptoms found were to be regarded as vestiges of pleuritis from the time during which the workers had been employed in the synthetic rubber industry or in other chemical industries has not been closely investigated. In view of the low incidence of pleuritis symptoms, such an investigation seemed to be of little interest, especially as it might be expected that the information given by the workers on the subject would be rather vague. Even with due reservation for the possibility that slight pulmonary changes may have been overlooked in the mass radiography films, these x-ray examinations seem anyhow to have shown that the workers in the Swedish synthetic rubber industry had not been affected with pleuritis to any greater extent, and at any rate not oftener, than in the other chemical industries with which comparison had been made.

About a year before this mass miniature radiography, x-ray pictures of the lungs and heart has been taken as regards six of the workmen in the synthetic rubber industry. These men represented a specially selected material, in that all of them were greatly affected with thoracic pains, dyspnea and fatigue. Apart from basal pleural adhesions in two of these workers, no undoubted pathological findings could be observed in the lungs. As regards

the heart, the x-ray photographs showed that the cardiac volume lay within normal limits and that no pathological changes had occurred in the heart contours.

### E. Electrocardiography in connection with hypoxemia test.

As at the pilot plant, the workers at the factory in question complained chiefly of fatigue and thoracic symptoms. The intense sensations of pain and pressure over the chest which several of the workers suffered from had, in some respects, a character resembling angina pectoris. They usually ensued after some exertion, and more often in cold than in warm weather. The feeling of pressure was usually localized in a region extending like a belt over the thorax on a level with the heart, whilst the pains were referred to the region below the sternum, from the larynx down to the epigastrium.

Though the experiments on animals had not shown any disturbance of the coronary circulation in connection with exposure to chloroprene, it nevertheless seemed desirable to study the workers' electrocardiograms in connection with hypoxemia and "work" tests. The question as to the superior advantage of the one test or of the other for diagnostic purposes seems still to be open. BRÖCK (1946) considers the hypoxemia test superior to the work electrocardiogram, but stresses the importance of performing both tests, as they do not completely cover one another. LARSEN (1938) prefers the work electrocardiogram to the hypoxemia test; EVANS and BOURNE (1941) are likewise of the same opinion.

In the hypoxemia tests, the present author caused the workers to breathe a mixture of 10 per cent oxygen and 90 per cent nitrogen for 20 minutes. The gas mixture was supplied to the tested subjects through a Lovén valve while they were resting on a couch. The electrocardiograms were taken with an electrocardiograph (Elmqvist system), the usual three extremity leads being obtained synchronously. The electrocardiograms were recorded (1) during rest before the test began and (2) immediately and (3) 10 minutes after its termination. As previously stated, the workmen's symptoms were particularly marked after the end of the working day, but afterwards, generally speaking, subsided rather rapidly. The men were

therefore asked to come for testing directly after they had stopped work. Fifteen of the workers most affected were subjected to hypoxemia tests. With two exceptions, all of them were employed in the distillation department. Twelve of the workers examined were between the ages of 20 and 35 years, two of them between 36 and 40, and one 47 years old. Generally speaking, the tests could be carried out without any appreciable subjective symptoms in the workers. One of them complained of pains in the chest, but they were not so marked as to prevent the test being carried to completion. Analysis showed that all of them had normal electrocardiograms during rest, nor could any pathological change of the electrocardiogram after the hypoxemia test be observed. In connection with some of these tests, determinations of the oxygen content in the arterial blood was made, in order to control the degree of hypoxemia.

#### **F. Functional test with bicycle ergometer.**

As for the "work" electrocardiograms, they were taken in connection with a load on a cycle ergometer, the oxygen consumption, cardiac rhythm and ventilation being recorded according to the technique for heart-and-lung function tests proposed by WAHLUND (1945), to which the author will revert further on. The test for each worker was taken on two different occasions, viz. (1) at the end of a weekly shift and (2) before a new shift was to begin, pending which the worker had been free from factory work for 48 hours. In this way it was possible to make a comparison between the worker's condition after a relatively long exposure, when his symptoms were usually pronounced, and after a time of rest, when the symptoms as a rule had markedly subsided. It was in fact of interest to ascertain whether there was any difference at these times of observation, particularly with respect to the said heart-and-lung function test, but also as regards the "work" electrocardiograms. In the tests three loads on the ergometer were applied, viz. 600, 900 and 1,200 kg.m. per minute. Each load was continued for 6 minutes and the transition to a higher load was made without any interval.

In these tests the author used a cycle ergometer provided with a brake-strap, both ends of which were severally fastened to a spring dynamometer. By tightening the brake-strap, the required



load was read as the difference between the deflections of the two spring dynamometers. The rate of work was kept practically constant by instructing the tested subject to follow the pace set from a metronome. The speed was further controlled by an electric recorder, which registered the number of revolutions on the brake-strap wheel. The speed of the wheel was 162 r.p.m. at a pedalling rate of 54 per minute.

Ten of the fifteen workers previously examined with the hypoxemia test were tested also with the above technique. All of them were between the ages of 20 and 35 years. Generally speaking, this test caused the men greater trouble than the hypoxemia test. In one case it had to be interrupted after a few minutes, as the tested subject was unable to continue because of severe pain localized in the region behind the sternum. This occurred after the worker in question had been exposed to chloroprene for about one week. On another occasion, when the same man had been freed from factory work for 48 hours, the test could be carried out without much difficulty. Three other workers in the course of the test reported pains with a similar localization, but they were described as very mild and did not occasion any discontinuance of the test. In all cases these symptoms were noted only at examinations made after the workers had been exposed to chloroprene at the factory.

Electrocardiograms were taken (1) before the test, (2) immediately after the test, and (3) 10 minutes after its termination. On comparison between the electrocardiograms taken during rest and after work it was found that the latter differed quite slightly from the former, and no changes that were certainly pathological could be observed. The reduction of the S—T segments was insignificant, not exceeding 2 mm in the three leads taken together, as regards any of the men examined. It is noteworthy that this was the case also with the worker whose pain during the test had been so intense that it had to be discontinued.

WAHLUND, in an investigation on a large number of military patients, examined the pulse rate at varying loads on a Krogh cycle ergometer. He recorded the rate after 2, 4 and 6 minutes at loads of 600, 900 and 1,200 kg.m. per minute, which had been driven in close succession for 6 minute periods. It was found that the difference in the pulse rate between 2 and 6 minutes under each load did not exceed 10 beats per minute in persons with healthy

heart and lungs. In case of a greater difference than 10 beats per minute, or if the pulse rate 10 minutes after the termination of the test exceeded its initial rate by 15 or more beats per minute, this was regarded as a token of insufficiency. In about 100 cases this test was combined by WAHLUND with a simultaneous determination of ventilation and oxygen consumption, with a view to obtain a gauge of the functional adaptability of heart and lungs at different loads.

As the cardiac minute-volume, according to several investigations (NEWBURGH and MEANS 1915, BOOTHBY 1915, LINDHARD 1915, COLLETT and LILJESTRAND 1924, and others), stands in linear relation to the oxygen consumption, an indirect gauge of the former can be obtained by measuring the latter. In calculating the ventilation coefficient for oxygen (the ratio between the ventilation and the oxygen consumption, reckoned in litres per minute) at the three different loads, WAHLUND found that the difference between these values at the highest and lowest load was insignificant in healthy persons, amounting at most to about 9 per cent. A difference of over 15 per cent is considered by WAHLUND to be pathological, but he points out that due consideration must be paid to the absolute value of the ventilation coefficient, which for healthy persons, in work of this kind, is less than 22. A figure over 24 would indicate a bad cardio-pulmonary function.

As pointed out above in connection with the "work" electrocardiograms, the author tested 10 workers in accordance with the above-indicated technique. The experimental procedure in these tests may, in part, be gathered from the statements made in connection with the electrocardiograms. A gauge of the ventilation was obtained by collecting in a Douglas bag the air expired during a certain time and then measuring it by means of a gas clock. The ventilation was computed at the end of each load period. Samples for air analysis, which was made in a Haldane apparatus, were obtained from the expired air in the Douglas bag. As previously mentioned, each worker was twice tested, viz. (1) after exposure to chloroprene during a week's work at the factory and (2) before a new weekly shift was to be started when the man had been free from factory work for 48 hours.

The result of this investigation is shown by the following table: —

**Table XVI. Test on bicycle ergometer of workers**

The tests were carried out 1) immediately after that the worker had finished his

Case	Department	Work — Rest	Puls rate before the test	Load I				
				Kg.m. min	Puls rate	Ventilation	Oxygen consumption	Coefficient of ventilation
P. O. G.	DIST.	W	72	530	112 112 126	18	1.16	15.5
		R	64	500	100 104 100	24	1.21	20.0
L. A.	DIST.	W	68	570	94 100 108	27	1.27	21.0
		R	56	530	102 114 114	20	1.06	19.0
O. B.	DIST.	W	88	530	108 102 110	24	1.12	21.0
		R	72	580	93 92 96	19	1.10	17.5
B. H. M.	DIST.	W	66	670	84 88 88	23	1.26	18.5
		R	74	590	86 92 98	24	1.56	15.5
R. S. G.	DIST.	W	64	500	84 92 90	19	1.18	16.0
		R	62	540	90 88 88	20	1.36	14.5
B. B.	POL.	W	78	520	116 118 118	16	0.88	18.0
		R	68	570	104 106 108	21	1.23	17.0
H. O. G. S.	POL.	W	76	560	104 104 104	23	1.20	19.0
		R	70	560	110 108 100	18	1.95	19.0
L. O.	POL.	W	72	540	92 98 102	25	1.03	24.0
		R	72	580	104 106 106	27	1.29	21.0
E. O. H.	POL.	W	66	570	100 96 108	20	1.11	18.0
		R	86	540	116 124 128	22	1.21	18.0
W. F.	POL.	W	56	560	96 92 92	22	1.17	19.0
		R	64	540	84 92 92	19	1.03	18.5

Firstly, as regards the difference between the 2 and 6 minutes value for the pulse rate at the different loads, it exceeds in some cases 10 beats per minute, but as a rule quite slightly. The difference during the first load should be judged with some caution, as the 2 minutes value, at any rate in some cases, is higher than would correspond to the actual work performed. Some of the workers were in fact affected by some nervousness at the start, which doubtless conduced to accelerate the pulse rate. Some minus differences during the first period can probably be explained in this way. The acceleration of the pulse during the second and third load periods seem to be more correct indications of the greater effort.

at the distillation and polymerization departments.

work for the shift (W), and 2) after that he had been free from work for 48 hours (R).

Load II					Load III					Puls rate after the test
Kg.m. min	Puls rate	Ventilation	Oxygen consumption	Coefficient of ventilation	Kg.m. min	Puls rate	Ventilation	Oxygen consumption	Coefficient of ventilation	
949	134 140 140	26	1.67	15.5	1 290	150 150 162	33	1.93	17.0	74
1 150	120 122 124	28	1.69	16.5	1 250	132 132 134	34	1.84	18.5	68
970	128 126 128	31	1.89	16.5	1 220	134 136 140	34	1.91	18.0	68
1 020	118 120 126	30	1.52	19.5	1 160	128 132 138	41	2.09	19.5	68
930	128 118 128	28	1.43	19.5	1 250	146 164 162	38	1.89	25.0	88
1 030	122 124 128	31	1.76	17.5	1 350	150 156 160	40	2.12	19.0	86
1 050	104 108 108	30	1.82	16.5	1 140	124 128 130	40	2.24	17.5	66
960	106 120 118	28	1.58	17.5	1 290	140 140 146	39	2.46	16.0	78
960	94 108 100	24	1.59	17.5	1 160	120 118 122	33	2.02	16.5	60
940	108 108 110	22	1.66	13.0	1 340	122 124 126	35	2.29	15.0	69
890	124 130 140	32	1.97	16.0	1 240	146 154 156	42	2.63	16.0	74
940	114 118 122	25	1.47	17.0	1 250	134 140 140	30	1.81	16.5	72
980	114 122 120	23	1.08	21.0	1 240	136 132 144	45	2.29	19.5	80
1 000	102 112 120	36	1.90	19.0	1 440	125 140 140	37	1.97	19.0	72
930	114 114 120	31	1.45	21.0	1 200	124 128 132	37	1.97	19.0	82
970	110 112 116	32	1.55	20.5	1 310	116 122 128	35	1.69	20.5	80
970	122 128 122	25	1.54	16.0	1 330	138 134 140	33	2.01	16.5	76
960	132 130 136	29	1.49	19.5	1 240	140 158 162	31	1.84	17.0	100
980	104 104 104	30	1.62	18.5	1 280	128 128 124	45	2.21	20.0	64
970	108 104 104	29	1.60	18.0	1 190	124 124 120	35	1.90	18.5	64

To judge by these functional tests, it is noteworthy that the performance of the workers after exposure to chloroprene seems to have been fully on a level with their capacity before it, though the subjective symptoms during the tests were more marked directly after the exposure. The ventilation coefficient in no case exceeded the figure 24 and, generally speaking, it was less than 21. In those cases where the ventilation coefficient showed a rise in connection with a larger load it was rather insignificant, in no case exceeding 10 per cent, reckoned according to the values at the lowest and highest load. With the criteria by which this test is being judged, it thus seems that the lung-and-heart function of the workers lay within normal limits.

Taken into account the results from the experiments on animals the author in connection with the above-mentioned two occasions of work-testing estimated also the oxygen content in the blood of the workers. There was no noteworthy difference, however, to be found between the determinations obtained at these two occasions.

## CHAPTER XVI.

### DISCUSSION.

The examination of the workers in the synthetic rubber industry in Sweden has clearly shown that the men employed in special departments of the factories were affected with certain symptoms of ill-health. Among the workers in the distillation department the symptoms were chiefly thoracic pains, fatigue and irritability, among those in the polymerization department chiefly loss of hair. The clear delimitation of the symptoms to workers employed only in these two departments indicated, with a high degree of probability, that they were attributable to the effects of chloroprene and its oxidation products or polymeric forms, as exposure to other substances did not occur there. To judge by the occurrence of the symptoms among the workers in relation to the manufacturing process, it seemed also probable that the cause of the loss of hair was to be found in the polymers of the chloroprene, whilst the thoracic pains as well as the fatigue and irritability were attributable to the oxidation products of chloroprene.

As regards the special effect of the polymers of chloroprene on the hair, it has not been subjected to close investigation in connection with this work. Here it may suffice to point out that, as betokened by many indications, the effect of the polymers is not due to an external local action on the hair or scalp, but to an effect probably occurring first after the resorption of polymers. Otherwise it is difficult to explain that, despite very effective protection from the direct action of chloroprene on the hair, loss of hair nevertheless resulted when the workers were in air containing vapours of polymeric chloroprene. Certain investigations by RITTER and CARTER that are likely point out a local action of the polymers do not seem to the present author to be quite convincing.

The nature of the pains in phosgene poisoning is very similar to that found in exposure to chloroprene. As the author has previously mentioned, certain parallels may in fact be drawn between the pathology of phosgene and chloroprene poisoning as regards the lungs of animals. There are, however, also marked differences in this respect. The pulmonary changes in connection with chloro-

from the lungs or air passages. It is probable that the thoracic pains in phosgene poisoning proceed only a secondary and later effect. It has therefore been considered is known to be a specific lung irritant, and cardiac insufficiency is slight or non-existent during rest. Phosgene is a gas which they are as a rule aggravated after bodily exertion, whilst they are poisoning when other symptoms have passed off. In such cases region below the sternum and may persist long after a phosgene referred to CHASIS, 1944). These pains are usually localized in the such poisoning (for the literature on the subject, the reader is poisoning that thoracic pains are a very common symptom after for example, from studies of the symptomatology of phosgene either with a pulmonary or a cardiac origin of the pains. It is known, factors. It seems, however, most probable that we must reckon and a discussion of the etiology is bound to involve uncertain cardiopulmonary system, the cause of these symptoms is obscure default of positive findings from the different examinations of the regarded as one of the most serious symptoms among them. In among the workers. They were often very intense and were Quite special interest attaches to the cause of the thoracic pains

which had caused anemia in the experiments on rats. tion in the air was very high and probably on a level with that anemia were observed. Here, however, the chloroprene concentra- that injures manifesting themselves in the form of a secondary manifestation of subjective symptoms. It was only at the pilot plant recorded with the technique employed, but quite sufficient for the as a rule was not sufficiently high to cause changes that could be findings was that the exposure to chloroprene in the factories lack of correlation between subjective symptoms and objective relatively speaking, so few. The probable explanation of the apparent objective findings in the medical examination of the workers were, pain and pressure over the chest, it seemed surprising that the Considering the marked symptoms of fatigue and especially of

prene are mainly secondary to a failing heart action with stasis, and the direct effect as a lung irritant is of minor importance. It is in fact quite rarely that a person exposed to chloroprene is troubled by a cough, whereas this is a common symptom in persons suffering from phosgene poisoning. The pulmonary edema after exposure to chloroprene is also much less marked than in phosgene poisoning. If thus certain factors indicate a pulmonary origin of the pains, the reasons for refusing to accept such a view are, on the other hand, probably still more cogent.

In several respects, there are great resemblances between the pains after exposure to chloroprene and the pains due to angina pectoris. The location of the former pains is in many cases the same as that of the pains that may occur in angina pectoris. Moreover, the occurrence of the pains after bodily exertion and their subsidence during rest is characteristic also of angina pectoris. It was found that the pains occurred more often in cold weather, which is yet another characteristic of angina pectoris.

The supposition that the origin of these pains is comparable with that of the pains caused by angina pectoris is indeed gainsaid by the absence of electrocardiographic changes in the examination of the workers. It should be pointed out, however, that these examinations were made after the hygienic improvements at the chloroprene factories had proceeded so far that thoracic pains, with a few exceptions, no longer occurred among the workers. The negative result of these examinations, therefore, does not entirely conflict with the above supposition. But, in view of the symptom picture among the chloroprene workers, in whom the sensations of pain were usually connected with tachycardia and dyspnea, and with reference to the experiments on animals, which had shown a marked effect of chloroprene on the heart, the author considered it at any rate more probable that the origin of the pains was cardiovascular rather than pulmonary, although it does not seem possible at present satisfactorily to explain their mechanism.



## CHAPTER XVII.

### CONTROL OF THE OCCUPATIONAL DISEASE HAZARDS IN THE SYNTHETIC RUBBER INDUSTRY OF SWEDEN.

Control of occupational disease hazards requires close cooperation between doctors and technicians. The collective experience from their different fields of work is, as a rule, essential for the maintenance of satisfactory hygienic conditions of labour. The primary measures usually develop on the doctor, whose attention is usually first drawn to the possibility that the work may be injurious to health. Not until the causes of the disease have been ascertained, can really effective protective measures be taken. The continued control of the occupational hazards will be largely a technical problem. This alone, however, never involves an adequate guarantee, but must be supplemented by a continuous medical control of the state of health of the employees. In an excellent work on this subject BRANDT (1947) writes: "By pre-employment and routine periodic medical examinations, by supervision of nutrition, diet and personal hygiene, and by education, the physician plays an important part in the prevention of occupational diseases through controlling the worker. Important as these measures may be, it is generally agreed that they are secondary to the control of the hazard. If the hazard is severe, control of the worker alone will either not prevent occupational illnesses or it will jeopardize competitive production. If, on the other hand, the exposure is controlled adequately, less-frequent periodic medical examinations and less-strict supervision of diet and personal hygiene will be needed."

Apart from occupational eczema and dermatites, most occupational diseases are caused by inhalation of noxious substances of some kind. One of the principal problems of occupational hygiene

will therefore be to endeavour to reduce the concentration of noxious substances in the inspired air to tolerable values. This "evaluation of the atmospheric health hazards" was in fact regarded as the central problem of occupational hygiene in the synthetic rubber industry in Sweden, when investigation had shown that the ill-health of the workers was attributable to chloroprene in the inhaled air. This work, however, met with great difficulties, chiefly owing to the lack of any satisfactory method for the determination of chloroprene in air. As previously indicated, the chlorine ion contained in chloroprene has but little tendency to reaction and cannot be freed even when heated with alkali in an aqueous or alcoholic solution. Only by the combustion of chloroprene can its chlorine be converted into analyzable form. This fact serves as a basis for the methods for the determination of chloroprene in air that have been devised by Russian authors. Owing to their elaborateness, however, these methods were found unsuitable for routine determinations. It was therefore necessary to test other alternatives. In the investigations taken up for this purpose at the laboratories of the synthetic rubber industry in Sweden, attempts were made in the first place to apply already available methods for the determination of hydrocarbons with a  $C=C$  double bond. The procedure that was found to be most suitable for the purpose was an iodine value method, which is based on the addition of a halogen to the double bond. In view of the slight reaction of the iodine molecule, however,  $ICl$  is used instead. The latter compound is easily formed in a solution of  $I_2$  in glacial acetic acid by the introduction of an equivalent amount of  $Cl_2$  (Wij's solution). In regard to this reagent, we know from its principal use, analysis of fatty substances, that the consumption is dependent on the excess remaining when the unsaturated compound has completely reacted. When the colour of the reagent changes merely quite slightly during the reaction, this method can be used solely in connection with a quantitative determination of the amount of halogen consumed. As the results of the analysis were found to be dependent on the manner in which Wij's solution is produced, a description of the way in which it was prepared for use in these tests is given below: —

13 g of pure iodine in pulverized form were dissolved in 1,000 ml of pure glacial acetic acid, with heating to  $80-90^\circ C$ . From the solution 100 ml was withdrawn for later use. Chlorine, having first bubbled through water and then through concentrated sulphuric acid, was caused to pass through

the major part of the iodine solution until the brown colour had changed to orange, when the supply of chlorine was at once discontinued. The non-treated iodine solution was now added to the major part of the solution until a faintly brown colour remained, indicating merely a slight excess of free iodine. The solution was heated at 80—90° C for 20 minutes.

The solution, which should be kept in a dark-coloured bottle with a ground-in stopper, must not be brought into contact with moisture; all vessels must be perfectly dry before use. By diluting this solution with glacial acetic acid, a suitable concentration, — reckoned according to the amount of chloroprene to be determined —, can be obtained. In the analyses made at the factory premises, a 0.1-n solution was used.

In taking the air samples, a burette for 1,000 ml gas, with a stop-cock at the top and bottom, was employed. Air from the place under examination was sucked through the burette for about 5 minutes, whereupon the cocks were closed. Through the lower cock, 10 ml of a 0.1-n ICl solution, prepared in the way above described, was supplied under pressure, after which shaking proceeded for 15 minutes. Afterwards 10 ml chloroform and 10 ml of a 10 per cent potassiumiodide solution were supplied through the upper cock. After further shaking, the content of the gas burette was introduced quantitatively into a flask and titrated with 0.02-n thiosulphate solution. Seeing that 1 molecule of chloroprene, as we know, adds 1 molecule of ICl, the amount of chloroprene in the air sample could be easily reckoned. The sensitivity of the method was found to be  $\pm 0.05$  mg chloroprene per litre of air at the chloroprene concentrations that occurred in the factories. At higher concentrations its sensitivity is less.

In this connection it seemed to the author to be of practical interest to compare the least concentration of chloroprene in air which still can be perceived with the sense of smell with the concentration that involves danger to health. Chloroprene, in view of its very characteristic smell, seems to be well suited for such a determination. In computing the threshold value of chloroprene, definite amounts of the substance were introduced into flasks with a capacity of 2 litres. When the chloroprene had completely evaporated, a number of persons (ten) were asked to inform us when the smell was still noticeable at falling concentrations of the chloroprene. In order to eliminate errors due to self-suggestion, "blind specimens" were here and there inserted in the series of chloroprene flasks.

Under these experimental conditions, it was found that concentrations of over 1 mg of chloroprene per liter of air were distinctly perceived by all the tested persons. Concentrations between

0.5 and 1 mg per litre of air were noticeable to most of them, whereas at amounts of 0.5 mg per litre of air merely one or two persons perceived the smell.

It is noteworthy that such relatively high concentrations of chloroprene were required to ensure that the characteristic smell of chloroprene should be perceived. As shown by statements made towards the end of this chapter, however, the concentration of chloroprene that involves danger to health is considerably higher than the least concentration perceptible by the sense of smell.

Before discussing the air analyses from the different factory premises, an account will first be given of the hygienic measure adopted in the pilot plant, and which had to be carried out without the aid of air analyses, as the above-mentioned method for the determination of chloroprene in air had not been elaborated at the time when operations were proceeding there. As the process of production could not be definitively designed from the outset, but underwent radical changes according as the technical procedure was improved, the hygienic situation in this plant, and thus also the hazards for the workers, showed frequent changes. This was manifested, for example, in a relatively large number of cases of acute poisoning, with giddiness and nausea. These symptoms, however, as a rule rapidly subsided.

In view of the limited space available in this factory and its character as a pilot plant, it did not seem possible, by more extensive technical measures, to reduce the risks for the workers. Only where the conditions under which the semi-mechanical operations were carried on were manifestly unsanitary, was this defect redressed. This was the case especially as regards the storage and transportation of chloroprene within the factory premises, for which purposes open vessels were often used. A very risky stage in the work was found to be the cleansing of apparatus and pipings. They had often to be cleansed from the polymerization products of chloroprene. This work was as a rule performed while the pipings were still hot, which entailed a marked evaporation of chloroprene and its polymers into the premises. On computation of the chloroprene concentration in the air close to the place where such operations were carried on in one of the larger factories, it appeared that it was about 4 times higher than the normal concentration elsewhere in the premises. In the sequel it was found necessary

to cool the piping and to ventilate it with fresh air before the work was performed. When this was not possible, the workers were recommended to wear fresh-air masks. Such masks were made obligatory also in all work in containers, in polymerization pots and similar places, where high chloroprene concentrations might be expected. Apart from these measures, which, though easily arranged, proved to be by no means unimportant, no more radical sanitary precautions, for the above indicated reasons, could be adopted. Occupational prophylaxis in this plant thus came to be very largely dependent on the ability of the workers themselves to avoid needless exposure. Experience has shown that the education of the worker for his own protection is a very important part of occupational prophylaxis.

It was found, however, that it was not sufficient merely to give the workers written instructions regarding measures of safety or, by notices posted up in the factory premises, to draw their attention to hazardous stages in the work: personal guidance and directions also proved to be necessary. The workers were made thoroughly acquainted with the manufacturing process and received detailed instructions as to the best way of protecting themselves from exposure to chloroprene. In the continued supervision and control required for the purpose, excellent work was done by engineers and specially appointed representatives of the workers.

The effect of these different measures was noticeable in successively diminishing symptoms of disease among the men. Cases of acute poisoning — except those which were attributable to accidents of some kind —, were completely eliminated. At the latest medical examination of the workers at this plant the author found merely a few cases of thoracic complaints. Compared with the incidence of disease during the early stages of this manufacture (see Ch. XIV), the improvement was thus very considerable.

On the other hand, it was evident that the hygienic measures adopted could not completely eliminate the disorders by which some of the employees were affected. In default of air analyses, merely very approximative estimates of the chloroprene concentrations in this plant could be made. In view of the general hygienic conditions in the plant and the marked symptoms among the workers, it may be presumed that the chloroprene concentrations, at any rate during the early stages, exceeded the maximum in other factories.

This is in fact indicated by the cases of acute poisoning, with giddiness and nausea.

At investigations subsequently made<sup>1</sup>, in which experimental subjects were kept for some time in a closed room where chloroprene was allowed to evaporate, giddiness and nausea set in only when the chloroprene concentration amounted to 3.5 mg per litre of air. The symptoms manifested themselves after about 15 minutes' exposure to this concentration when the subjects were at rest, seated on chairs; but were already noticeable after 5—10 minutes if they performed light physical work. Such high chloroprene concentrations, however, doubtless occurred merely at some few places in the plant and for a comparatively short time. As soon as the above-mentioned precautionary measures had been adopted, the chloroprene concentrations appear to have been considerably reduced both at the said places and within the premises as a whole.

In planning the larger factories, endeavours were made, in the light of the experience from the pilot plant, to make provision for better hygienic conditions. In several cases special "control rooms" were erected, from which hazardous processes in the work could be controlled. So far as possible, attempts were made to arrange the entire process of production within a self-contained system. In order to reduce the above-mentioned risks in the cleansing work, the apparatus was duplicated at suitable places, so that the work could be arranged in shifts. In this way, the work could be carried on under less risky conditions, whilst the occasional stoppages that had previously occurred were avoided.

As already pointed out, the state of health among the workers at these newly started factories, despite all the sanitary improvements adopted, was not satisfactory at first. Investigation showed (see Ch. XV) that the workers in the distillation and polymerization departments were extensively affected with symptoms of disease. An improvement had, however, set in, seeing that no symptoms of acute poisoning ever occurred in these factories. It may therefore be presumed that the chloroprene concentration in the inhaled air never amounted at any stage in the work, even for a short space of time, to 3.5 mg per litre of air.

The first determinations of chloroprene in the air of the distilla-

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<sup>1</sup> For assistance in this investigation, the author is indebted to Dr. Johan Wersäll.

tion and polymerization departments were made in the spring of 1946, when the above-described method of analysis had been so thoroughly tested that it could be applied in practice.

It is often difficult to select time and place for the analysis of air samples so that the values found shall be representative of the risks of exposure in a factory. This presupposes a thorough knowledge of the course of the production cycle and of the time required by different stages in the work. So far as such high concentrations of noxious substances occur at the place of work that they involve a risk of acute poisoning, they should in the first place be limited in time and space and, as soon as possible, be completely eliminated. Otherwise, the risk involved by a high concentration obviously cannot be judged until the time of exposure to it is known; and the high value in itself should not determine the sanitary measures to be adopted.

In many cases it is only the "weighted average atmospheric concentration" that gives a true indication of the exposure. This concentration is computed in accordance with the formula

$$\frac{E_1 T_1 + E_2 T_2 + E_3 T_3 + \dots + E_n T_n}{T_1 + T_2 + T_3 + \dots + T_n},$$

where  $E$  represents the concentration for a given substance in the air at  $n$  different operations, and  $T$  the time required for carrying out the operation in question (BRANDT).

The chloroprene concentrations in the inhaled air, expressed in Fig. 18 below, refer to the places in the polymerization and distillation departments where the workers must remain during the major

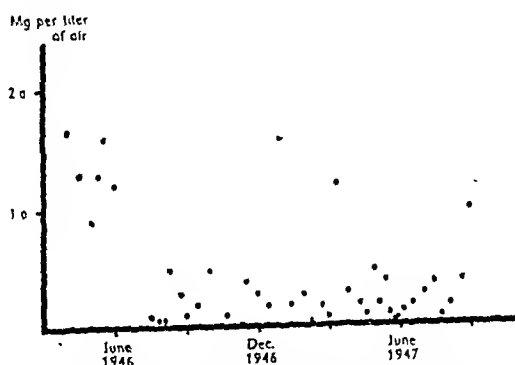


Fig. 18. — The concentration of chloroprene in the air before and after effective improvements of the ventilation system.

part of the working-hours, and where the figures may be expected to be higher than in other parts of the premises. To what extent fluctuations of the chloroprene concentrations had occurred within the daily exposure time has not been closely investigated in these first samplings. The values found were, in fact, generally speaking, so high that even if certain reductions of the chloroprene concentrations may have occurred, they would in any case have occasioned improvements in the hygienic conditions.

As it seemed scarcely possible to make the general ventilation more effective within the factories, endeavours were made, instead, to reduce the amounts of chloroprene concentrated in the air by means of local exhaust devices at the places in question, besides further concentration of the manufacturing process.

Renewed air analyses were made from the autumn of 1946, when the above-mentioned precautionary measures had been adopted and operations had been resumed to their full extent. The analysis expressed in Fig. 18 relate to the period from August 1946 to September 1947.

Apart from some relatively high chloroprene concentrations, the cause of which could in general be ascertained, the other figures show that the concentrations had been considerably reduced, as compared with previous analyses. As the high values relate to comparatively short stages in the work at which moreover fresh-air masks were obligatory, it seems that only the other figures need be discussed from a toxic point of view.

On the basis of experiments on animals, v. OETTINGEN et al. point out that chloroprene concentrations down to 0.3 mg per litre of air may cause toxic injuries in man. According to reports from the Du Pont Company, it was the rule there that the inhaled air should not contain a larger amount of chloroprene particles than 0.1 mg per litre of air. The same figure is given by BRANDT. To judge by the present author's own inhalation experiments on animals, the risk limit should lie somewhat over 0.2 mg chloroprene per litre of air. It seems thus reasonable to accept a limit value of 0.3 mg chloroprene per litre. This would imply that the chloroprene concentrations during the first observation period were throughout much too high. The analyses from the later observation period show, on the other hand, that most of the figures lie below this limit and merely a few slightly above it.



The improvement in hygiene thus indicated was in fact reflected, as previously pointed out, in a decrease of symptoms of disease among the employees. At the end of the last observation period the state of health was found to be very satisfactory, and the author, at a medical examination then made, did not note any symptoms worth mentioning among the workers.

## Summary.

This investigation deals with the following questions:

1. Are there any indications that the work in the synthetic rubber industry injuriously affects the health of the employees?
2. What substance or substances or stages in the process of production can be considered to cause deterioration in health?
3. Do experiments on animals with the substance or substances in question show that they are injurious to the animal organism?
4. Is there any correlation between the observations from experiments on animals and the symptoms found among the workers?
5. What measures can be taken to reduce the risks of ill-health in this industry?

*1. Are there any indications that the work in the synthetic rubber industry injuriously affects the health of the employees?*

- a) In examination of the workers in this industry, the author found (1) a group who complained mainly of thoracic symptoms, fatigue and irritability, and (2) another group who had no other noteworthy symptoms beyond falling off of the hair.
- b) These symptoms affected only the workers in certain departments of the factories. The first-mentioned symptom picture was found only among workers employed in the chloroprene distillation department, the last-mentioned only among workers in the mass polymerization department.

*2. What substance or substances or stages in the process of production can be considered to cause deterioration in health?*

- a) As the workers in the distillation department were exposed only to pure or oxidized chloroprene and in the polymerization department to polymeric forms of chloroprene, it seemed that in

all probability the symptoms of the workers were caused by those qualities of chloroprene.

- b) It has been shown that phosgene, which is often formed on the decomposition of chlorinated hydrocarbons, is not produced from chloroprene even under very favourable conditions.
- c) In order to obtain a most complete knowledge of the substances in question it was made an investigation into the effect of polymerization on the rate of evaporation of chloroprene, and the solubility of chloroprene in water was determined. In this connection the author gives a brief resumé of the chemical constitution of chloroprene and its polymers as well as their chemical and physical properties, besides a survey of the production of artificial rubber in accordance with the synthetic chloroprene process.

3. *Do experiments on animals with the substance or substances in question show that they are injurious to the animal organism?*

- a) The experimental investigations on animals comprised (1) mortality determinations in long-continued and shorter tests, and (2) studies on the effects of chloroprene on the heart, lungs, kidneys, the central nervous system and the blood.
- b) In inhalation tests on rats which had been exposed to 1.2 mg of chloroprene per litre of air for 8 hours daily during 5 months, five out of ten of the animals died within the course of 13 weeks, whereas at a concentration of 0.2 mg all the ten rats survived the whole exposure period.
- c) In studies of the effects of chloroprene on isolated rabbit's and frog's hearts, it was shown that concentrations down to 0.002 ml per 1,000 ml of Tyrode's solution respective 0.01 ml per 1,000 ml of Ringer's solution had a distinct depressant effect on the heart.
- d) In inhalation tests on rabbits, a marked fall of the arterial blood pressure ensued, whilst the pressure in the right auricle increased. This effect is explained as a manifestation of a heart action similar to that found in the tests on isolated hearts.

No changes in the electrocardiograms could be observed in connection with these tests.

- e) On the subcutaneous injection into rats of oxidized chloroprene in doses from 0.000125 to 0.008 ml per gram of the body weight, the result was an increase of the lung weights, which was found

to be due to hyperemia with pulmonary edema. At the highest dosage the lung weight in percentage of the body weight amounted to 1.3, as against normally 0.7—0.8. No similar effect could be observed in the injection of non-oxidized chloroprene.

In inhalation tests with oxidized chloroprene at a concentration of ca. 17 mg per litre of air for 5 hours, the lung weight amounted to 1.6 per cent of the body weight, as against 0.9 per cent in the control animals.

- f) In urea clearance tests on rats, it was noted that after inhalation of 5 mg oxidized chloroprene per litre of air for 6 hours, the clearance value fell to about 50 per cent below normal.
- g) The effect of chloroprene on the central nervous system was studied with a modified Knoefel-Murrell technique. It was found that different qualities of chloroprene differed considerably in narcotic effect. It was most marked in the case of oxidized chloroprene. It was also shown that the narcotic effect increased with the degree of polymerization.
- h) The inhalation tests on rats with a concentration of 1.2 mg chloroprene per litre of air for 8 hours daily during the course of 5 months resulted after some time in the development of a secondary anemia, besides a moderate leucocytosis. With a concentration of 0.2 mg chloroprene per litre of air, but otherwise under the same experimental conditions, no blood changes could be observed.
- i) It has been shown that the oxygen content of the blood was reduced in connection with the exposure to chloroprene, which may be attributed to a simultaneously observed decrease in the oxygen capacity of the blood.
- k) In inhalation tests on rats a statistically significant decrease in the coagulation time after the exposure to chloroprene was noted.
- l) In inhalation tests on rats the author noted a statistically significant increase in the hematocrit value after the exposure.

4. *Is there any correlation between the observations from experiments on animals and the symptoms found among the workers?*

- a) To give an answer to this question examination of the workers was made on repeated occasions in the course of the years 1944

—1947, at first comprising all the men employed in the factories, but according as the symptoms were found to be limited to workers in the aforesaid two departments only, the examination was concentrated mainly on those workers. These inquiries comprised (1) a general bodily examination, (2) laboratory analyses of the blood and urine, and (3) certain special investigations.

- b) The bodily examinations showed, broadly speaking, a very satisfactory state of health among the workers and the few diseases observed had as a rule been previously known to them and could not be attributed to their work in the rubber industry.
- c) The laboratory investigations showed an anemia of secondary type among the workers from one of the factories, where the men had at first been exposed to a particularly high concentration of chloroprene. According as the chloroprene concentration in the air was reduced, this anemia subsided. Otherwise the laboratory analyses were negative.
- d) The special investigations were the following: —
  - 1. Mass miniature radiography of the lungs.
  - 2. In a smaller number of cases x-ray examination of the heart and determination of the cardiac volume.
  - 3. Electrocardiography in connection with hypoxemia and work tests.
  - 4. Determination of the Schneider index.
  - 5. Cardiopulmonary function tests with a cycle ergometer.
  - 6. Determination of the basal metabolism.
  - 7. Renal function tests according to Rehberg's creatinin clearance.
  - 8. Determination of the liver function with hippuric acid and thymol tests.

None of these special investigations gave evidence of pathological changes or disturbances of function that could be attributed to the work in the synthetic rubber industry.

- e) Thus in the investigations made merely a few objective findings were noted, which seems surprising in view of the results from the experiments on animals and the marked subjective symptoms among the workers.

5. *What measures can be taken to reduce the risk of ill-health in this industry?*

- a) In considering the precautionary measures to be adopted for this purpose, the principal problem seemed to be to reduce the chloroprene concentration in the inhaled air to a tolerable value.

To judge by the toxicity tests on animals, the value that can be tolerated by the human organism seems to lie at about 0.3 mg of chloroprene per litre of air.

- b) The air analyses in respect of the chloroprene concentration were made in accordance with a method elaborated at the factory laboratories.
- c) Various technical measures, such as improved ventilation devices, self-contained systems of production, separate premises for control of the operations, have been taken in order to reduce the concentration of chloroprene within the premises where the work was being carried on. The effectivity of these measures has been checked by repeated air analyses.

*Finally, as a result of the various hygienic improvements in the factories, the symptoms of disease among the workers have been almost completely abolished.*

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CORRECTIONS TO BE MADE IN  
*ACTA MEDICA SCANDINAVICA SUPPLEMENTUM CCXIX*

- Page 6, line 14, for Electrocariography, read: Electrocardiography.  
" 14, " 1, from below, for chloroprene, read: polychloroprene.  
" 19, " 7, from below, for far, read: for.  
" 21, in the foot-note, for author present, read: present author  
" 43, line 9, for oxydized, read: oxidized.  
" 51, " 9, for courses, read: course.  
" 58, " 15, from below, for ethyloxide, read: ether-oxygen.  
" 64, Table VII, case no. 16, for 4.47 read: 2.47.  
" 66, line 9 & 11, from below, for ureal nitrogen, read: urea nitrogen.  
" 100, " 4, for Electrocardography, read: Electrocardiography.  
" 110, " 9, for develope, read: devolve.  
For carotic artery, in the text, read: carotid artery.



# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CCXX (220)

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## THE DISTRIBUTION OF RED BLOOD CELL DIAMETERS IN LIVER DISEASES

AN INVESTIGATION OF THE MATURATION  
OF THE ERYTHROCYTE

BY

*GERHARD LARSEN*

M 8

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# THE DISTRIBUTION OF RED BLOOD CELL DIAMETERS IN LIVER DISEASES

AN INVESTIGATION OF THE MATURATION  
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BY

GERHARD LARSEN

*To speak of statistics as the study of variation also serves to emphasise the contrast between the aims of modern statisticians and those of their predecessors. For, until comparatively recent times, the vast majority of workers in this field appear to have had no other aim than to ascertain aggregate, or average, values. The variation itself was not an object of study, but was recognised rather as a troublesome circumstance which detracted from the value of the average.*

(R. A. Fisher)





## FOREWORD

The present investigation is based mainly on observations made while I served as assistant in the medical wards of the Norwegian Deacons' Hospital, Oslo, from January 1943 to October 1946. A few supplementary observations, some of the experiments, and all the work relating to pernicious anaemia, were performed while I was serving as assistant in the Medical Department A, University Hospital, Oslo.

I wish to express my heartfelt gratitude to my former chief, Olaf Bang M. D., and to Professor Olav Hanssen, M. D., for their unfailing interest in my work, for valuable advice and many helpful suggestions, and for their permission to use the material.

I am particularly grateful to cand. act. John Lotherington who has been my teacher and adviser in statistics. Without his many valuable suggestions and his helpful criticism this investigation could not have been carried out. All the statistical work has, however, been done by the author, who is solely responsible for any faults which may become apparent.

To medical student Gurly Gleditch and to deacon Johan Wilhelmssen and deacon Th. Seierstad, I wish to tender my thanks. They were my assistants during the first period of the investigation, when the time-consuming experiments, to develop a measuring technique, were carried out. I am deeply grateful for the zeal and exactitude with which they worked in spite of the seemingly hopeless results we steadily obtained.

My thanks are also due to Professor Ch. Finbak and his assistants for valuable advice on the questions of physical nature discussed in chapter IV, and to Dr. Kaare Heiberg for much advice and technical help in questions relating to photography.

The arguments used in the discussion in chapters X—XII, and the conclusions arrived at, are mainly based on the frequency distribution of the red blood cell diameters in blood films taken from the patients mentioned in table 46. It has been impossible to reproduce all frequency curves (about 450 in all) here. Four complete sets of these curves have however been prepared and deposited in the library of the Medical Dept. A, University Hospital, Oslo.

Towards this investigation, I have received grants from Doctor Alexander Malthes Fund, from Direktør Gotfred Lie og Hustru Marie Lies Fund, and from Grosserer Thor Dahls Fund.

The translation from Norwegian to English has been carried out by Mrs. Sheilagh Stene.

University Hospital. Medical Dept. A.  
Oslo — Norway, August 1948.

*Gerhard Larsen.*

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## Notation.

The notation is that used in current haematological and statistical literature, but some symbols and abbreviations may be explained:

A/G-ratio (A/G)	Albumin/Globulin ratio in serum.
Corp. vol. (C.V.)	Mean corpuscular volume of red blood cells in cub. my.
d. f.	Degrees of freedom.
Hb.	Haemoglobin in g pr. 100 ccm blood.
I. I.	Icteric Index (Meulengracht).
m	arithmetic mean.
$m_o, m_l, m_r$	When dealing with heterogeneous curves, the affix $_o$ designate the main component, the affix $_l$ the left component, and the affix $_r$ the right component.
MCH	Mean corpuscular haemoglobin in g . $10^{-12}$ .
MCC	Mean corpuscular haemoglobin concentration in %.
MD	Mean diameter of the red blood cells.
MT	Mean corpuscular thickness.
my	1/1000 millimeter.
N	Sum of frequencies.
$N_o, N_l, N_r$	Significance of affix: see m.
P	Probability. When relating to $\chi^2$ , the value refers to data in tables given by <i>Fisher</i> (1) pg. 112—113 and by <i>Bonnier &amp; Tedin</i> , pg. 324—325. When relating to $v^2$ , the value refers to data in tables given by <i>Mather</i> pg. 260—263 and by <i>Bonnier &amp; Tedin</i> pg. 318—321.
R	Reticuloocyte count in %.
R f.	Red cell fragility.
R. bl. c.	Red blood cell count in millions pr. cub. mm.
$\sigma$	Standard deviation of the population.



s	Standard deviation of a sample.
$s_o, s_l, s_r$	Significance of affix: see m.
$s^2$	Variance.
$s_b^2$	Variance between classes.
$s_w^2$	Variance within classes.
$s_t^2$	Variance of total.
SR	Sedimentation ratio (mm in one hour).
T	Treatment (T:O = Symptomatic treatment only).
Tk	Takata-Aras reaction.
$v^2$	Variance ratio (The ratio of two estimated variances).
$v^{2*}, v^{2**}, v^{2***}$	The asterics indicate the probability-levels and refers to the tables for P. (* = P : 0.05, ** = P : 0.01, *** = P : 0.001).
Vol %	Haematocrit value.
Xray	(in table 46) Cholecystography.

## CHAPTER I.

### Introduction.

LEEUVENHOECK, who invented the microscope and discovered the blood cells was, according to GROEN, the first person who tried, in 1674, to measure the diameter of the blood cells. Later, JURIN, HALE, and others, tried similar measurements but it was not until the middle of the last century that the microscopie technique had become so advanced that the measurements gave more or less concordant results.

The first attempt to determine the volume of the red blood cells was made by HARTING (1859), WELCKER (1864) and v. LIMBECK (1896) and was later, by a number of authors, developed into the now so well known haematoerit-method.

In 1889, HAYEM suggested to use the index colorimetricus as a measurement for the size of the blood-cells, and in 1919 PIJPER re-discovered that the red blood cells' average diameter could be determined by diffraction (halometry, erimetry), a method which for the first time was described by TH. YOUNG in 1823.

The size of the red blood cells can thus be determined by four different methods. By halometry one can determine the blood cells' average diameter, by the haematoerit method and by the determination of index colorimetricus, one gets an expression for their average volume. But there is only one method which also gives information on the distribution of the single blood cell diameter around the mean. That is the method where the diameter of a number of blood cells is measured and then the average diameter is calculated.

## Direct measurement of the blood cells' diameter.

The methods are numerous. One can measure the blood-cells suspended in plasma, serum, or in another diluting fluid (the «wet method») or one can measure the blood cells in a blood-film, stained or unstained, (the «dry method»). The measurement can be done with a micrometer in the ocular of the microscope, or one can first reproduce the blood cells in known enlargement, by drawing or by photography, and then measure the size on the drawing or photograph. It lies outside the limits of this work to give a detailed historical account of the different methods. Such accounts have been given by PRICE JONES, JØRGENSEN & WARBURG, MOGENSEN, GÜNTHER, HERNBERG, and others. Here

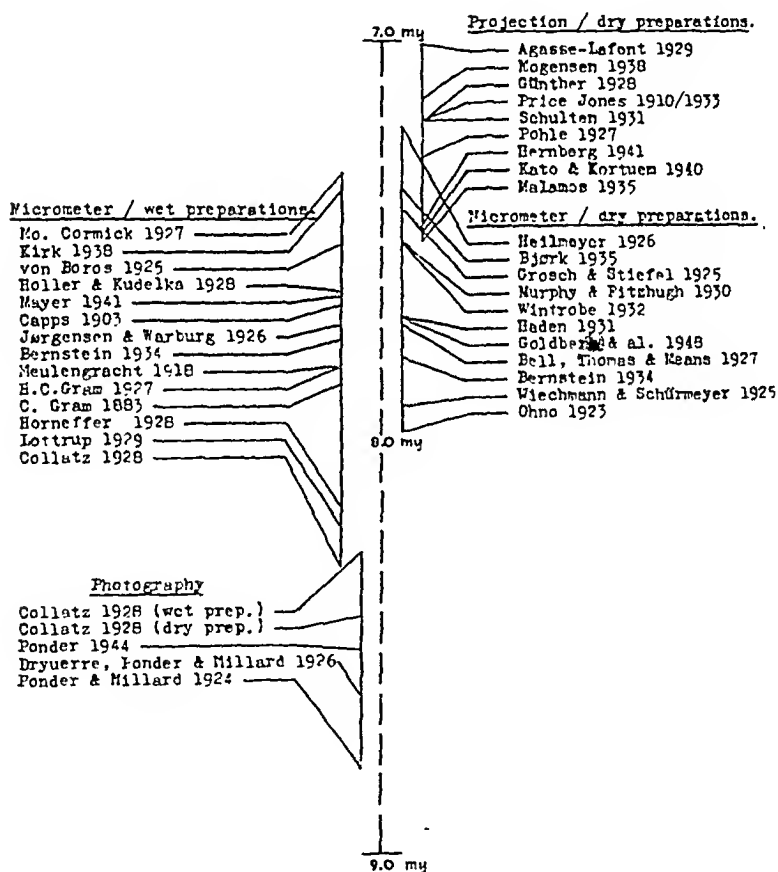


Fig. 1: Normal values of MD as determined by different authors using different techniques.

we shall only mention a few facts with relation to that which follows.

If one looks through the literature, one finds that the different authors' reports on the blood cells' normal diameter vary considerably, from 7.0 my to 8.82 my. This difference is not due to the fact that the different authors have examined people of various races (RICHARDSON, WISCHNEWSKY), neither can difference in sex, (OHNO, PRICE JONES, v. BOROS) nor differences in age (PRICE JONES, HERNBERG), explain the divergencies. It is usually assumed that the difference between the various authors' normal values is due to technical factors and factors peculiar to the individual investigator. In Fig. 1 are shown the normal values of the blood-cells' diameter, as given by several authors. It is obvious that these normal values divide themselves into groups according to the applied method, but even within each group there are considerable variations between the individual authors' values.

### *The personal errors.*

That such errors do occur is directly shown by LOTTRUP and WARBURG who by measuring the same preparation found a difference of 0.4 my between their results. Fig. 1 shows that there is a difference of 0.16 my between the normal value of v. BOROS and MAYER and of 0.20 my between the normal value of BELL, THOMAS' & MEANS and MURPHY & FITZHUGH, and in both instances they report that they are using exactly the same method. The personal factor seems to play the greatest role when the investigator uses the ocularmierometer. Possibly because this method is extremely taxing on the eyes (H. C. GRAM (1), HORNEFFER), but also possibly because interference between the blood-cells' edge and the mierometer's scale give rise to considerable subjective judgment. (BOCK & JOMBRES, PONDER (1)).

### *The technical errors.*

These are of different nature, depending on the preparation, the opties, and many circumstances which are only partly investigated. *In the wet preparation* the blood cells' are largest when they are suspended in their own plasma or serum, smaller when they are suspended in Hayem's liquid or Tyrode's liquid (OHNO).

The use of anticoagulants may change their size, and prolonged storage may also change their diameter (JØRGENSEN & WARBURG). Whether spreading and drying plays any role is not quite decided. Already THS. YOUNG has reported that the blood-cells did not change their diameter by spreading and this is later reported by LAACHE, WIECHMANN & SCHÜRMEYER (1), VON BOROS (2), GÜNTHER (2), OHNO, BERNSTEIN, GOLDBERG and others. On the other hand, PONDER, PONDER & MILLAR, PRICE JONES (2), MANASSEIN and others report that the blood cells decrease in size when dried, while COLLATZ finds that their diameter increases. The question can not be regarded as finally settled. The staining and fixing does not seem to influence the size of the blood cells. (GÜNTHER (2), BERNSTEIN, LEPEL, OHNO).

The optics employed in the microscope are of definite importance. GÜNTHER (2) and later MOGENSEN (1), HERNBERG, LEPEL, and LINDGREN have shown that the blood cells seem smaller when one employs oil-immersion-objective than when one uses dry-lense-objective. The difference is between 6% and 10%. HAMMARSTEN (3) suggests that this is caused by refraction-phenomena at the cell edge, but I have not found any adequate explanation of the phenomenon in previous literature. KAEMERERS information that the results differ if one uses the plane or concave mirror of the microscope fits in here.

It seems therefore, as if apparently minor details may play an unexpectedly important role. In addition it must be considered that the individual authors do the measuring in different ways. Thus, PRICE JONES, MOGENSEN and others measure the blood cells' largest and smallest diameter and take the average of these two measurements as the diameter of the cell. Others measure only the largest diameter (Mc. CORMICK) or a chance diameter (all methods using ocularmicrometry). Some measure with a scale graduated to  $\frac{1}{2}$  my (PRICE JONES, MOGENSEN), others draw the blood cells on millimeter paper and count the squares (HERNBERG). Others again measure the projected picture directly with dividers (GÜNTHER). And finally, some measure the area of the blood cells and not their diameter (MANASSEIN, AGASSE-LAFONT).

Since the method gives so varying results from one investigator to another, several authors (WINTROBE (3), HADEN (1), PONDER (1), deny its practical value and prefer instead the determination

of mean corpuscular volume by the haematocrit method. PRICE JONES (1), MOGENSEN, HERNBERG, GÜNTHER, VON BOROS and others admit that the results certainly vary, but maintain that it gives a relative value which is so exact that the method has its justification. As previously mentioned, the direct measurement is the only method which gives information on the composition of the blood cell population, or the degree and nature of the anisocytosis, and PRICE-JONES, MOGENSEN and TÖTTERMAN among others, have shown that this information may be of considerable importance.

### *Practical importance.*

The first practical result was obtained by the measurement of the blood cells in cases of anaemia. In 1864, WELCKER mentioned for the first time that the blood cells are smaller than normal, microcytosis, in chlorosis, a sign which later was particularly stressed by HAYEM in 1889 and CAPPS in 1903. Large blood cells, macrocytosis, in pernicious anaemia are first mentioned by SØRENSEN in 1876 and pointed out as an important sign by LAACHE in 1883. PRICE JONES, VAUGHAN, GOODHART and others have during the years 1910—1934, in a series of publications stressed the importance of the characteristic distribution-curves of blood cell diameters in pernicious anaemia, in the differentiation between this form of anaemia and other macrocytic anaemias. The question regarding the size of the blood cells has, during later years, once more been brought to the fore by American authors (WINTROBE, HADEN and others) who try to group the anaemias according to the blood cells' mean volume, and by European authors who suggest a similar classification based on the average diameter measured by halometry (BOCK, VON BOROS and others).

### **Macrocytosis in liver disease.**

C. GRAM is, as far as we know, the first who in 1883 pointed out the increase in blood cell diameter in 8 patients with jaundice. Similar discoveries were made by ENGELSEN in 1884, by VAQUEZ in 1897, and by CHAUFFARD in 1907.

v. LIMBECK is in 1896 the first to mention that the volume of the blood cells increases in jaundice, whereas, HAYEM (1889) and PERRIN (1908), are the first to find an increase in index colorimetricus in patients with liver diseases.

The phenomenon is later described by several authors. *The increase in the blood cell diameter* is shown by measurement of blood cells in plasma by MEULENGRACHT in 1918, GAMNA in 1926, by VON BOROS, H. C. GRAM, HOLLER & KUDELKA, JØRGENSEN & WARBURG, all in 1927, by SCHULTEN & MALAMOS in 1932, MALAMOS 1935, ARCHI 1936, RODIÑO & BAÑOS 1940, HAMMARSTEN (1) 1942, and HAMMARSTEN & STÄHLE 1943, by measurement of blood-cells in blood films by GÜNTHER in 1928, VAN DUYN (1933), MOGENSEN (1938) and LINDGREN (1947).

*By halometry:* CHENEY 1933, BOCK 1934, LUCKNER & TILGER (1936), SCHALM (1937) and BOCK & JOMBRES (1939).

*Increased mean corpuscular volume in jaundice* is mentioned by WINTROBE & SCHUMACHER (1933), SCHULTEN (1933), GROEN (1935), WINTROBE (6) 1936, and ROSENBERG (1936), and *increased index colorimetricus* in liver diseases is reported by VAN DUYN (1933), FELLINGER & KLIMA (1934), WRIGHT (1934), SCHIÖDT (1) (1935) and BENHAMOU & NOUCHI (1937). This list is hardly complete.

Macrocytosis appears also *in other digestive diseases:* In pancreatic diseases (CHENEY, HOLLER & KUDELKA) in ventricular diseases (BENTZEN, GRÉGOIRE, GORKA, VAUGHAN (2), and in ileitis (PLUM & WARBURG).

Several authors (CHENEY, HOLLER & KUDELKA, BENTZEN, SCHALM) presume that the same mechanism is the cause of this phenomenon in all these circumstances. It is worth mentioning in this connection that EFSKIND reports occasional considerable regressive changes of the liver parenchym in patients with serious digestive diseases.

*Experimentally caused macrocytosis in liver diseases:* HIGGINS & STASNEY poisoned rats with carbon-tetrachloride and HEINLE, CASTLE & ROSE caused, in the same way, poisoning in white mice. On both occasions the experimental animals developed liver cirrhosis and macrocytic anaemia.

### *Occurrence.*

Several authors are of the opinion that macrocytosis occurs in practically all cases of serious liver disease. SCHULTEN thus sees the phenomenon in 100 %, MALAMOS in 24 out of 26 cases, SCHALM in 100 %, HAMMARSTEN in 100 % and ROSENBERG in 91 % of all cases with serious liver disease.

On the other hand, there are several authors who maintain that the phenomenon admittedly occurs, but that it is relatively rare. WINTROBE & SCHUMACHER find it in 25 %, VAN DUYN in 18 %, WRIGHT in 27.4 %, BENJAMOU & NOUCHI in 25 %, FELLINGER & KLIMA in 65 % and GROEN in 25 % of the cases with serious liver disease.

With the exception of ROSENBERG, all authors who are of the opinion that the phenomenon occurs frequently, have measured the mean diameter of the blood cells, while the authors who maintain that the phenomenon is rare have measured the blood cells' mean volume, either by the haematocrit method or by calculating index colorimetricus.

This phenomenon, that the diameter increases, whereas the volume remains normal, is found directly by parallel researches by ARCHI and SCHULTEN (3, pg. 237), and is also mentioned by DEDICHEN.

The significance of this has not been fully clarified, but one has to reckon with the possibility that two different conditions are present: firstly, «macroplany», increase of the diameter without a simultaneous increase in the cells' volume, and secondly, possibly less frequently, macrocytosis with increased volume.

Most authors who have investigated the blood cells' volume have, by the way, not investigated the blood cells' size in liver diseases, but the size of the blood cells in anaemia during the course of liver diseases. Thereby, they are bringing in an important new factor whose significance has not been clarified.

LANGE (1), and LEICHSENBRING, DONELSON & WALL have shown that there is a negative correlation between the number of the blood cells and their average volume, and ISAACS (2) points out that there are two types of anaemia, which occur in connection with liver cirrhosis, one macrocytic and one microcytic.



### *Significance.*

SCHALM, ARCHI, HAMMARSTEN, LINDGREN, ODIN and others are of the opinion that the mean diameter is normal in jaundice due to occlusion without simultaneous liver disease.

SCHALM therefore suggests using the mean diameter determined with halometry as a diagnostic method, a suggestion which, by the way, first was made by VAN DUYN in 1933. HAMMARSTEN and her collaborators state in a series of publications, that the distribution curve of the blood cell diameters, i.e. the degree of the anisocytosis, is very different in the different liver diseases. They think they can draw far-reaching diagnostic and prognostic conclusions from the appearance of the distribution-curve. The question regarding the size of the blood cells in liver diseases thereby assumes a considerable practical interest. When the method after all has not come into common use, it is mostly due to the great amount of time which has to be spent on the examination. But it is also important that we, for the time being, deal with clinical observations whose mechanism is not sufficiently well known. If the method should find practical use the reasons which cause the phenomenon ought to be clarified.

### **The cause of macrocytosis in liver disease.**

The cause of macrocytosis in liver disease is not yet clear. The explanations which have been offered fall into two groups: Several authors are of the opinion that the already circulating blood cells swell and increase — «the theory of the peripheral disturbance». Others state that the liver disease causes a change in erythropoiesis so that the new blood cells which are sent out from the bone marrow are larger than normal, the «theory of the central disturbances».

### *Theories of «the peripheral disturbance».*

The earliest investigators — C. GRAM and MEULENGRACHT — were of the opinion that jaundice as such was the real cause of the macrocytosis and that the degree of macrocytosis was in direct proportion to the degree of jaundice. A great number of later authors (CAPPS, ROSENBERG, MALAMOS, BOCK, SCHULTEN,

HAMMARSTEN, MILLER & AL, LINDGREN, and others) have not been able to find such a correlation. It is also difficult to accept jaundice as a cause, when the opposite of macrocytosis — microcytosis — is a typical characteristic of haemolytic jaundice, and since SCHALM, HAMMARSTEN, ARCHI and others have shown that the phenomenon does not occur with jaundice due to occlusion. HOLLER & KUDELKA have further shown that macrocytosis may appear in liver diseases without jaundice, and ROSENBERG, HAMMARSTEN and others have shown that macrocytosis may remain after the jaundice has disappeared.

Those who assert the theory of «the peripheral disturbance», base their opinion on two arguments: MOGENSEN, SCHALM, ARCHI and TOTTERMAN point out that the macrocytosis appears and disappears so fast that a disturbance in the bone-marrow, according to their opinion, can not be the cause.

MOGENSEN thus found an increase in the MD of 0.43 my over the normal upper limit after 26 days, and an increase of 0.35 my over the normal upper limit after 12 days, in two cases of acute hepatitis, and C. GRAM found a decrease of 0.167 my after 25 days and 0.093 my after 9 days, during the course of acute hepatitis. CUENEY, BOCK, MOGENSEN and SCHALM also state that the increase of the mean diameter is not followed by an increase of the anisoeytosis. The change in the mean diameter must therefore, they maintain, be due to changes in the size of *all* blood cells. And should this change be due to an altered erythropoiesis, the blood cells' life time would have to be considerably shorter than usually assumed. But since these patients with acute hepatitis do not develop any considerable anaemia, and since there is no sign of increased regeneration in the form of reticulocytosis, polychromasia or the appearance of nucleated red blood cells, one can not assume any such shortening of their life-time. The macrocytosis must therefore be brought about by a change in the size of the already circulating blood cells. The central point in this argumentation is that there is no increased anisoeytosis in the peripheral blood. But it must be stressed that there is no full agreement on this point. Already C. GRAM pointed out that these patients had an unusual number of large blood cells, and HAMMARSTEN, LINDGREN, and GAMNA stressed just the anisoeytosis as a characteristic feature in the blood picture in liver diseases.

The other argument for the theory of the peripheral disturbance is an experiment which was first made by ENGELSEN. ENGELSEN mixed normal blood cells with icteric plasma and noticed that the mean diameter of the blood cells increased by 0.34  $\mu$ . He did not however, obtain any reduction in the size of the pathologic blood cells when these were stirred out in normal plasma. C. GRAM and MEULENGRACHT could not reproduce ENGELSEN's experiment.

V. LIMBECK, HAMBURGER, PRICE JONES (7) and WIECHMANN & SCHÜRMEYER are of the opinion that the red blood cells' diameter increases after exercise, in diabetic acidosis, in venous congestion, in heart failure, and in other conditions accompanied by an increase of the blood's  $\text{CO}_2$  content. HOLLER & KUDELKA presume that this is also the cause of macrocytosis in liver diseases.

Acidosis is however no constant phenomenon in liver diseases, and ROSENBERG and MOHR find no correlation between the size of the blood cells and the degree of acidosis in diabetic coma. Based on careful experiments DRYERRE, MILLAR & PONDER maintain that the blood cells do not change their size with increased  $\text{CO}_2$  content in the blood as assumed by the above-mentioned authors.

GAMNA, ROSENBERG, BETHEL & ROTTSCHAEFER, RODINO & BAÑOS and others, are of the opinion that macrocytosis is due to a change in the serum albumin/globulin ratio with subsequent change in the colloid osmotic pressure. And finally, BANG & ØRSKOW and BETHEL & ROTTSCHAEFER believe that the permeability of the blood cells is changed. GÜNTHER regards a change in the surface tension and JOLLY dehydration, as the cause of the changes in size.

BRÖCH (1 Page 66) and MAIZELS & WHITTAKER maintain, based on experimental studies, that osmotic disturbances can not be the cause of the phenomenon.

### *Theories of «the central disturbance».*

Against the above-mentioned theory of disturbances in the peripheral blood there have been raised a series of objections which at the same time are arguments for the view that the cause lies in the bone marrow. HADEN (5) and later GUEST & WING have

shown that the red blood cells in vitro behave like perfect osmometers. They attract water when suspended in hypotonic salt solutions. Their volume increases until they become spherical, and they thereby have the largest possible volume for a given surface. When this point has been reached they disintegrate and haemolysis occurs.

HADEN (1) and WINTROBE (3.5) therefore argue that if the changes were due to osmosis the volume would have to increase with the 3rd power of the mean diameter. But such an increase in volume has, as mentioned before, not been observed.

CAPPS is the first to point out that if the swelling of the blood cells is caused by an absorption of water, the saturation-index would become smaller and the mean corpuscular haemoglobin concentration would decrease. Therefore, since several authors find an increase in index-colorimetricus in liver diseases this does not fit in with the assumption of the peripheral cause. VON BOROS (3.4), FELLINGER & KLIMA, and SCHULTEN & MALAMOS report as well that the blood cells in liver diseases contain more haemoglobin than they do normally. MOGENSEN finds a decreased haemoglobin content, but points out that his patients had anaemia.

### The bone marrow in liver disease.

If macrocytosis in liver diseases should be due to a changed erythropoiesis, one might expect to find changes in the bone marrow in these patients. Such changes have also been observed by several authors:

BLEICHROEDER and ROSSIER at autopsies invariably found red bone marrow in the long tubular bones where there normally should be fatty marrow. The marrow had also undergone qualitative changes. TISCHENDORF and KIENLE find an increased number of all cellular elements. ROSSIER mentions an increased activity in the erythropoiesis with a simultaneous decrease in the thrombopoiesis, and ISAACS finds an increased number of erythroblasts with a tendency to macrocytosis. ALDER finds in humans, and FREERKSEN (3) in animals, a macrocytosis in the bone marrow with simultaneous macrocytosis in the peripheral blood. Finally, DAVIDSON & McCRIE, PERSON, and FITZMUGH & PERSON state

that the reticulocytes are larger than normal in patients with macrocytosis in the peripheral blood.

HOLLER & KUDELKA and BECHER assumes that the macrocytosis is due to a toxic effect on the bone marrow from toxins produced in liver disease. HAMMARSTEN & STÄHLE suggest the possibility of a toxic effect on a haematopoietic centre in the diencéphalon (also suggested by PINEL, HORTLING and ASK-UPMARK), but otherwise the common opinion is that the macrocytosis is due to a lack of — or lacking utilization of — the antipernicious principle of the liver. This last assumption is so common that it is stated as a fact in a number of newer textbooks in haematology. (WINTROBE (7), HADEN (8), KRACKE, WHITBY & BRITTON).

It may therefore be proper to compare liver diseases and pernicious anaemia.

### Liver disease and pernicious anaemia.

*Clinically* one does not observe in liver diseases the usual symptoms of pernicious anaemia with any regularity. Neither achylia, glossitis, nor myelopathia are usual symptoms, and seldom or never are the high-grade anaemias seen (CASTLE & MINOT).

*The blood findings* are also different. The constant increase in the mean volume of the blood cells is not seen in liver diseases, neither is the marked anisocytosis, poikilocytosis or polychromasia (SCHULTEN (3 pg. 237), WINTROBE (6)). The typical changes of the leucocytes in the peripheral blood, and the typical changes in the bone marrow of untreated pernicious anaemia are never seen in liver disease (SCHULTEN, SCHULTEN & MALANOS).

*Therapeutically* one sees no effect of liver extract on the macrocytosis in liver diseases (CHENEY, FELLINGER & KLIMA, GOODHART). A frequently cited case observed by GOLDHAMMER, where a patient with liver cirrhosis had an increase in haemoglobin from 48 % to 64 % in 63 days after having had altogether 60 ccm. liver extract, does not seem very convincing.

SCHIFF, RICH & SIMON and GOLDHAMMER, ISAACS & STURGIS have produced a fully potent liver extract from cirrhotic human liver and thereby shown that these patients do *not* lack the antipernicious principle. This in contrast to liver extract made from patients who died with untreated pernicious anaemia. Such an ex-

tract is completely ineffective (WILKINSON & KLEIN, RICHTER, IVY & KIN).

CASTLE & MINOT, DEDICHEN, MALAMOS and WATSON & CASTLE therefore maintain that another principle than the anti-pernicious principle must be the cause of the changes in the bone marrow. This opinion is expressed thus by CASTLE & MINOT (Page 13):

*«certain chemical entities must be present in order for the normal transition from immature to adult red blood corpuscles to take place in the bone marrow. Specifically, it is probably necessary for the body to have available a certain active principle found in liver and certain other tissues, which is effective in pernicious anaemia, in order for the megaloblast to progress to the normoblast. For maturation of the normoblast into the adult red blood cell, iron, another active principle found in liver, and probably in other animal organs, copper, vitamin C and thyroxin are probably all essential.»*

It is not known which substance this «another active principle found in liver», is. But the investigations by GOODHART, WILLS, and VAUGHAN (1) seem to indicate that the principle may be found in the vitamin-B complex.

CASTLE & MINOT's book dates from 1936. Since then folic acid has been discovered and studies of the haemopoiesis with new methods, as use of radio-active iron (ROBSCHERT-ROBBINS WHIPPLE, HAHN & CO-WORKERS), quantitative analysis of the single cell (THORELL), mitosis research (KIENLE and others) and other methods have brought all these problems into a new light. These new results have been published while the present investigation was in progress, and have not to any great extent influenced the working plan. They will therefore not be discussed here, but in the concluding chapter, where my results are compared with them.

There are thus two theories which may explain the macrocytosis in liver diseases. Both theories apparently have decisive arguments in their favour, but they exclude each other as well.

The arguments for the theory of disturbances in the bone marrow: The lacking increase in volume, the red blood cells' increased content of haemoglobin, and the changes seen in the bone marrow, appear to me to be so important that this theory

probably is correct. But before this theory can be accepted, a satisfactory explanation of the fact that the macrocytosis appears and disappears so fast, as shown by MOGENSEN, SCHALM, ARCHI and TÖTTERMAN, must be given.

And if such an explanation is found, it remains to be shown if the macrocytosis is due to the same cause as in pernicious anaemia.

PRICE JONES (6) indicates a method which may solve the problem. He first mentions that a certain amount of anisocytosis of the red blood cells normally occurs, and mentions that the anisocytosis can be expressed graphically by drawing frequency curves according to the method he has developed. He then says:

*«I suggest that in conditions associated with marrow dysfunction the curve is shifted to right or left and the distribution is more or less asymmetrical or skew. In those conditions in which the cells are acted on by some common factor in the circulation, and only indirectly or secondarily associated with disordered haemopoiesis, the variability is more frequently normal or relatively slight, and the distribution is more or less symmetrical.»*

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## Résumé of Chapter I.

### The problem. The working plan.

After a short description of the methods which are at our disposal for determining the size of the blood cells, direct measurement of the blood cells by micrometry is discussed. It is shown that the method has many sources of error, partly of an unknown nature. The literature on the size of the blood cells in liver diseases is reviewed. It is shown that macrocytosis appears in liver diseases, and that it is probable that the diameter is more often increased than the volume of the blood cells.

The theories which have been proposed to explain the phenomenon are mentioned. These theories fall into two groups, the theories of the peripheral cause, and the theories of a changed erythropoiesis.

*The following questions are unanswered:*

1) *When does macrocytosis appear in liver diseases? How fast does the phenomenon appear and disappear?*

2) *Is the macrocytosis due to changes in the peripheral blood or is the cause a changed erythropoiesis?*

3) *Has the macrocytosis in liver diseases the same cause as macrocytosis in pernicious anaemia?*

4) *If the increase in MCV in liver diseases is not due to the same cause as in pernicious anaemia, what is then the cause of the phenomenon?*

5) *Which diagnostic and prognostic conclusions may be drawn from the presence of macrocytosis, and which methods can be used when the phenomenon is to be utilized in the clinic?*

These questions, all or several of them, may be answered by following a number of patients with liver diseases with frequent measurements of the size of their blood cells during the whole course of the disease. In order to answer several of the questions it is not sufficient to determine the mean size of the blood cells. It is necessary to determine how the single blood cell diameters are distributed around their mean.

The only method which is then left is some form or other for direct measurement of the blood cells. But in the introduction it is shown that these methods all have considerable and mostly unknown sources of error. Before these methods can be utilized for the solution of the above-mentioned questions the cause of the errors must be determined, so that the errors as far as possible, can be eliminated.

*The present work therefore falls into two parts:*

*Part one* must contain a detailed analysis of the measuring technique in order to determine its reliability, both in the measurement of normal and pathological blood samples.

*Part two.* If a satisfactory technique can be developed it may be used on a clinical material in order to answer the questions.

These problems, in both parts of the work, are largely of statistical nature and one will therefore, to a great extent, use modern



statistical methods. But the use of statistics assumes that one operates with factors which can be measured, weighed or in other ways expressed in figures. I will therefore, in the present work, not make use of methods which largely have to be based on personal judgment. One may particularly mention two methods which will *not* be used:

*Routine use of sternal marrow puncture* will not be made. Several of the previously mentioned questions might possibly be answered by studying the bone marrow in these patients. But practical experiments have convinced me that I, neither by differential counting, nor by measurement of the diameter of the cells from the sternal marrow, can obtain such accurate results that they can stand critical statistical investigation.

Neither will I use *puncture biopsy*.

Puncture biopsy in liver diseases has its justification in enabling a correct diagnosis. This can as a rule be achieved, if not equally fast, by clinical observation. In the few cases where the clinical observation did not result in a diagnosis explorative laparotomy with biopsy has been performed.

## CHAPTER II.

### Statistical methods.

#### *Frequency distributions.*

All blood cells which at a given moment are circulating in the organism constitute a *population*. From this population individual blood cells are steadily disappearing, dying, and new ones are steadily added from the bone marrow. Not all blood cells are of equal size. A certain degree of anisocytosis is physiological.

When one measures the diameter of a large number of blood cells, the values found are ranged in classes according to size (fig. 2 a). Each class is called a *variate class* or *variate*. In fig. 2 a. 6.00, 6.25 . . . . . 8.75 thus represent variate classes. The number of blood cell diameters in each variate class is called *frequencies*. In the example 3, 2, 10 . . . . . 1, thus are frequencies. The sum of all frequencies is called the *frequency sum* and is designated by the letter N. The arithmetical mean of all frequencies is the sample's (blood cells') mean diameter which is designated by the letters MD. The distribution around this mean is measured by the *variance* which is the mean of the squares of deviation of the frequencies from the mean. The positive square root of the variance is called the *standard deviation - s* - which is another measure for the distribution of the single diameters around the mean.

The frequency-distribution in fig. 2 a may be represented graphically: Measuring the variate-value along the x-axis and the frequencies along the y-axis one obtains a frequency polygon which gives a graphic picture of the distribution of the blood cell diameters (fig. 2 b).

The true value of the population's mean diameter and variance is unknown. All measurements can only give approximate values,

Class Mark	Tally Marks	Number of cells
6.00	///	3
6.25	//	2
6.50	//// ////	10
6.75	//// //// //	12
7.00	//// //// //// //// //// //	32
7.25	//// //// //// //// //// //// //// //// //	43
7.50	//// //// //// //// //// //// //// //// //	43
7.75	//// //// //// //// //	22
8.00	//// //// //// //	19
8.25	//// ////	10
8.50	///	3
8.75	/	1
Total		202

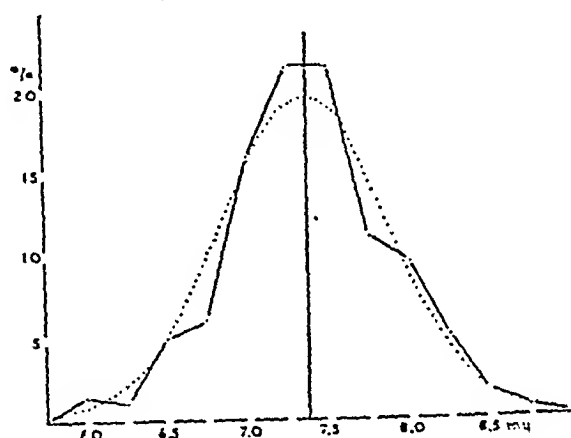


Fig. 2. a and b:

Frequencies, observed frequency curve, and corresponding normal curve.

which will deviate more or less from the true values owing to random sampling and technical errors. The values determined by actual measurements are called *characteristics*, the corresponding true, and unknown, values are called *parameters*. Even if the true value of a parameter can never be determined by actual measurements, one can, when one possesses a number of characteristics, determine a field, inside which the parameter must lie. The more characteristics one has, the more exact the determination of this field will be.

*$\chi^2$ -analysis.*

According to PRICE JONES' and GÜNTHER's investigations the blood cell diameters in normal subjects are distributed around the mean according to the normal curve. This normal distribution may be calculated when the MD and  $s$  are known (fig. 2 b). But fig. 2 b shows that the observed frequency curve and the calculated normal curve do not agree. This is due to random sampling errors. One can, therefore, for each variate class calculate the difference between the number of observed frequencies and the number of expected frequencies. Some of these differences will be positive, others will be negative. Since the frequency sum is the same in both curves, the sum of these differences will be zero. If, however, all the differences are squared, we get positive figures only, and the greater the single differences are, the greater the sum of these figures will be. One might expect that the difference which is due to random causes would be greater in variate classes with many frequencies than in variate classes with few frequencies. We therefore divide the difference in each variate class with the number of expected frequencies in the class, and the sum of these figures is called  $\chi^2$ .

*$\chi^2$  is therefore:*

$$\text{The sum of } \frac{(\text{expected frequencies} - \text{observed frequencies})^2}{\text{Expected frequencies.}}$$

The value for  $\chi^2$  depends upon the difference of the expected and observed frequencies in each variate class. But  $\chi^2$  does as well depend upon how many figures are added, in other words, the number of variate classes. The more variate classes, the greater  $\chi^2$  will be, under otherwise equal circumstances. In practice  $\chi^2$  is placed in relation to the number of *degrees of freedom*, or the number of classes whose frequencies can be chosen independently. If, for instance, we have 10 variate classes, and we know that the frequency sum should be 200, then the number of frequencies in the last variate class must be given when the frequencies of the nine others are determined. In this case there will therefore be 9 degrees of freedom. If, besides, the mean value of the sample is given in advance, then the frequencies of at least two variate

classes must be given when the others are determined. The number of degrees of freedom is then  $10 - 2 = 8$ .

If an experiment as shown in fig. 2, is repeated several times, the curves will not have the same appearance. Some will show a good and others a poor conformity with the normal curve. Some will therefore have a small, others a larger value for  $\chi^2$ . It is possible to calculate how these values for  $\chi^2$  will be distributed, and tables for this distribution have been worked out. When we have found a value for  $\chi^2$  we find in the table for the correct number of degrees of freedom the figure which comes closest to the observed value. A value  $P$  corresponds to this figure.  $P$  tells us how often one may expect an equally high or higher value for  $\chi^2$  if the experiment is repeated under identical conditions an infinite number of times. A value of 0.50 for  $P$  indicates therefore that if the experiment is repeated an infinite number of times one half of the results will be better and one half will be poorer than the present experiment. If we get a value for  $P \approx 0.001$  we are thereby told that if the experiment is repeated an infinite number of times we will obtain an equally high or higher value of  $\chi^2$  only once in a thousand times. It is then justified to assume that other causes than the purely random ones are to blame for the poor results.

### *Analysis of variance.*

If an experiment as shown in fig. 2 is repeated many times one will obtain a number of samples. For each sample one may calculate a mean diameter. These samples will not be exactly alike, but will differ somewhat owing to random sampling errors. Neither will the values for MD be the same for all samples. But it may be shown that if only random sampling errors play a role, then the observed values for MD will be distributed around *their* mean with a variance  $\frac{\sigma^2}{N}$ , where  $\sigma^2$  is the true variance of the whole population, and where  $N$  is the number of frequencies in each sample.

We have thus obtained two estimates for the true mean diameter: Firstly, the mean diameter in each sample, and secondly, the mean of all these mean diameters and their variance around

this mean. Finally, we may obtain a third estimate for the same value by adding all samples together into a grand total and calculate the mean for the whole collection.

These three estimates of the same value should agree if only random causes are of influence. Interplay between the single values can be measured in several ways. In the present work, one uses the *variance ratio* as a measurement for this interplay (see BONNIER & TEDIN, KENNEY, MATHER). Likewise as in the  $\chi^2$  analysis, the calculation of the variance ratio leads to the determination of P.

If one makes a series of measurements of the same blood sample with different techniques and undertakes analysis of variance of the results, a certain variance ratio is obtained, and this may be compared with the tables for the variance ratio and P. If the ratio, for instance, corresponds to  $P : 0.20$  it will mean that were the experiment repeated many times, 20 % of the results would be poorer than the one observed, only due to random sampling. The conclusion would be that the difference between the techniques might easily be due to random causes. If, on the other hand, the variance ratio corresponded to  $P : 0.001$ , it would mean that only one out of a thousand similar experiments would show equally poor conformity, and the conclusion would have to be that the differences in technique probably influenced the result. The variance ratio therefore gives an expression for the probability that an observed difference is due to random causes. We speak about the 5 %, 1 % and 0.1 % limit for this probability, and designate these limits with 1, 2 or 3 stars. A variance ratio with \*\* therefore means that the probability, that a difference is only due to random causes, is less than 1 : 100.

### *Random sampling.*

Where the population is known to be normally distributed, and where the true value of the mean and the variance is known, the theoretical curve for the population may be calculated. The observed curves can then be compared with the theoretical curve by  $\chi^2$ -analysis, so that one may obtain an expression for the «goodness of fit». In this way one may learn if the observed curves deviate more from the true distribution than allowed for by ran-

dom sampling errors. In other words, one gets a measurement of the magnitude of the possible technical errors.

Where the true distribution of the population is unknown, this method can not be used. One may, however, add together a large number of observations into a grand total, and presume that this grand total is the best expression of the true distribution of the population. In order to learn how much of the variation which is due to random causes, one may draw new samples from this grand total by random sampling. The frequencies so obtained can be grouped in classes and treated in the usual way. The distribution of samples so obtained will only be influenced by random sampling errors, whereas errors due to technical faults, faulty measurements etc. will be eliminated. To evaluate the importance of these technical errors one may then compare the samples obtained by random sampling from the grand total with the samples really obtained, using  $\chi^2$ -analysis, analysis of variance, or other means.

The random sampling can be made by writing out the frequencies of the grand total on paper slips, putting them in a hat and really drawing lots. But it may be done in a more practical way by using *random sampling numbers*, tables especially prepared for this purpose. In the present work, such tables prepared by TIPPETT have been used. For further details ref. TIPPETT, YULE & KENDALL and KENDALL.

In this work analysis of correlation, curvefitting and other statistical methods will also be used. We will here follow procedures described in the usual text-books of statistics.

## PART I

MEASUREMENT OF THE RED BLOOD CELL  
DIAMETERS

CRITICAL EVALUATION OF THE METHODS





## CHAPTER III

### Measurement by projection. The errors of the method.

We have shown in the introduction that the different methods for measurement of the red blood-cells' diameter has both «personal» and «technical» errors. In order to analyse these errors more closely it is practical to start out with one method — review it in detail, and then later investigate how it varies from other methods for measurement of the diameter of the blood cells. Fig. 1 shows that the method with projection of the blood films gives results which show the greatest conformity between different authors. This method has a series of practical advantages as well and it is therefore natural to choose it as a basis for the investigation.

#### Price Jones' method

as described in detail by MOGENSEN (1 p. 21), was employed with the following changes: The blood films were coloured with «May-Grünwald-Giemsa's stain» instead of the «Jenner stain». Instead of a projection prism, which could not be obtained because of the war, I used a Leitz drawing ocular. The microscope's degree of enlargement was adjusted to exactly 1000 x against a Zeiss objectmicrometer with a 10 my scale. The optical equipment of the microscope was an achromatic oil immersion objective 1/12 Leitz and a Leitz drawing ocular which enlarged 8 x. A random selection of blood cells was drawn with a sharp, hard pencil, and their size was then measured on the drawings. The largest and the smallest diameter were measured and their mean reckoned as the blood cell's diameter. Measurement was done with an accuracy of  $\frac{1}{2}$  mm, with scales specially prepared for the purpose. The same scales have been used throughout the whole investigation. Since the degree of enlargement is 1000 x,  $\frac{1}{2}$  mm corresponds to  $\frac{1}{2}$  my.

*The errors of the method.*

*Experiment no. 1:* In order to determine the errors, 5 blood films were prepared in rapid succession from a normal person. The five stained preparations were investigated by 3 different assistants. Each assistant measured 500 blood cells in each blood-film, distributed over 5 series, each of 100 blood cells. We had therefore altogether 75 series each of 100 blood cells distributed over 5 films and 3 assistants, with 75 determinations of the mean diameter and the standard deviation (table 1).

TABLE 1.

*Five blood films prepared in rapid succession from the same person.  
Mean diameters and standard deviations (in  $\mu$ ), as determined by three assistants  
drawing and measuring 5 series of 100 cells in each film.*

Blood film no.	Assistant		
	A	B	C
1	6.98 - 0.47	6.87 - 0.38	7.24 - 0.60
	7.04 - 0.53	6.73 - 0.39	7.15 - 0.53
	6.92 - 0.52	6.93 - 0.35	6.96 - 0.54
	7.10 - 0.56	6.90 - 0.38	6.98 - 0.52
	6.96 - 0.47	6.93 - 0.39	6.91 - 0.58
2	7.08 - 0.49	6.90 - 0.38	7.05 - 0.52
	6.95 - 0.49	6.87 - 0.36	7.26 - 0.59
	7.07 - 0.42	6.95 - 0.35	7.13 - 0.57
	7.13 - 0.41	6.89 - 0.39	6.93 - 0.52
	6.92 - 0.48	6.89 - 0.44	6.96 - 0.52
3	6.92 - 0.46	6.97 - 0.43	7.03 - 0.52
	6.93 - 0.39	6.94 - 0.37	7.09 - 0.61
	6.99 - 0.53	7.02 - 0.40	6.98 - 0.51
	6.82 - 0.47	6.99 - 0.35	6.92 - 0.55
	6.96 - 0.48	6.97 - 0.36	7.00 - 0.60
4	7.13 - 0.46	6.93 - 0.35	6.79 - 0.59
	7.06 - 0.47	6.94 - 0.36	6.75 - 0.56
	7.24 - 0.52	6.95 - 0.37	6.81 - 0.53
	7.20 - 0.47	7.00 - 0.39	6.98 - 0.51
	7.20 - 0.51	6.87 - 0.38	7.13 - 0.61
5	7.06 - 0.50	6.93 - 0.39	7.08 - 0.60
	7.10 - 0.52	7.00 - 0.40	6.87 - 0.53
	7.08 - 0.45	6.90 - 0.37	7.02 - 0.57
	7.15 - 0.45	6.85 - 0.34	7.01 - 0.50
	7.05 - 0.49	6.82 - 0.39	6.86 - 0.58

The analysis of variance (tables 2—3) shows that both the determination of MD and  $s$  varies more in this experiment than allowed for by random sampling. The analysis of MD shows a significant variance ratio between the determinations made in the different blood films, as well as between determinations made

TABLE 2.  
*Analysis of variance of data in table 1.*  
*Variance of mean diameters.*

Item	Degrees of freedom	Variance
Between assistants . . .	2	$s_B^2$ 0.0982
Within assistants . . . .	72	$s_W^2$ 0.0106
Between blood films .	12	$s_b^2$ 0.0247
Within blood films .	60	$s_w^2$ 0.0078
Total . . .	74	$s_t^2$ 0.0130

Variance ratio:

$$\frac{s_b^2}{s_w^2} : 3.17^{**} \quad \frac{s_B^2}{s_w^2} : 12.59^{***} \quad \frac{s_B^2}{s_b^2} : 3.98^*$$

TABLE 3.  
*Analysis of variance of data in table 1.*  
*Variance of standard deviations.*

Item	Degrees of freedom	Variance
Between assistants . . .	2	$s_B^2$ 0.1952
Within assistants . . . .	72	$s_W^2$ 0.0012
Between blood films .	12	$s_b^2$ 0.0008
Within blood films .	60	$s_w^2$ 0.0012
Total . . .	74	$s_t^2$ 0.0064

Variance ratio:

$$\frac{s_w^2}{s_b^2} : 1.50 \quad \frac{s_B^2}{s_W^2} : 162.67^{***}$$

by the different assistants. Whether this second difference is real is measured by the ratio:

$$\frac{s_B^2}{s_b^2}$$

which in this experiment is 3.98\* The ratio shows that the difference probably, but not definitely, is real. But when the analysis is altered so that «variation between the blood films» becomes the primary, «variation between assistants» the secondary cause of variation, this ratio becomes 1.06, which is not significant. Were the difference between assistants due to differences between the blood films, this ratio ought to have been greater. The conclusion must be that in this experiment, it is a real difference between the different assistants' determinations of MD.

When it concerns the *standard-deviation* the experiment is more definite. The variance ratios show a great certainty for a difference between the individual assistants' determination of the standard deviation, but no significant difference in the assistants' determination of the standard deviation in the different blood films.

#### *Errors due to measurement.*

*Experiment no. 2:* The variation between the individual assistants may be due to the fact that they *draw* the blood cells differently or that they *measure* them differently, or both. The personal errors in *measurement* must disappear if all measurements are done by the same person. The drawings which were prepared for experiment no. 1 were therefore remeasured by a fourth assistant (tables 4, 5, 6).

When drawings from experiment no. 1 are remeasured by a fourth assistant, the observed difference in experiment 1 between the individual drawers' determination of the mean diameter disappears. The measurement of MD must therefore be subject to causes of variation peculiar to each assistant, and these causes of variation must be connected with the *measurement* of the blood cells.

TABLE 4.

*Mean diameters and standard deviations (in  $\mu$ ) determined by assistant D when measuring the drawings from table 1.*

Blood film no.	Drawn by assistant		
	A	B	C
1	6.81 - 0.52	6.81 - 0.44	7.15 - 0.57
	6.82 - 0.56	6.96 - 0.43	7.01 - 0.53
	6.62 - 0.48	6.80 - 0.52	6.85 - 0.56
	6.87 - 0.56	7.05 - 0.45	6.99 - 0.53
	6.86 - 0.47	7.15 - 0.51	6.86 - 0.64
2	6.86 - 0.50	6.74 - 0.53	6.95 - 0.53
	6.83 - 0.54	6.76 - 0.44	7.15 - 0.49
	6.96 - 0.53	6.70 - 0.39	6.99 - 0.58
	6.99 - 0.43	6.79 - 0.49	6.82 - 0.52
	6.75 - 0.50	6.86 - 0.46	6.83 - 0.47
3	6.69 - 0.47	6.94 - 0.51	6.91 - 0.55
	6.78 - 0.48	6.75 - 0.42	6.96 - 0.56
	6.92 - 0.59	6.98 - 0.49	6.87 - 0.50
	6.63 - 0.56	6.92 - 0.41	6.82 - 0.58
	6.85 - 0.57	6.90 - 0.43	6.93 - 0.52
4	6.80 - 0.51	6.76 - 0.40	6.63 - 0.58
	6.97 - 0.52	6.76 - 0.44	6.57 - 0.53
	7.07 - 0.55	6.81 - 0.47	6.61 - 0.51
	7.13 - 0.51	6.83 - 0.44	6.76 - 0.51
	7.07 - 0.52	6.77 - 0.44	6.93 - 0.54
5	6.93 - 0.51	6.86 - 0.48	7.04 - 0.58
	6.95 - 0.51	6.97 - 0.41	6.86 - 0.58
	6.99 - 0.49	6.92 - 0.40	7.00 - 0.51
	7.06 - 0.52	6.74 - 0.40	6.99 - 0.51
	7.07 - 0.56	6.64 - 0.45	6.86 - 0.54

The difference between the single assistants' determination of the *standard-deviation* still remains when the drawings are measured by a new assistant, and the difference can therefore, on the whole, not be due to the measurement. But one may not from this conclude that the measurement is of no importance. If the standard deviations in experiment no. 1 are compared with the corresponding values in experiment no. 2, a statistical difference is found for the series drawn by assistants A and B. In these two series the act of measuring therefore influences the value of the standard deviation.

TABLE 5.  
Analysis of variance of data in table 4.  
Variance of mean diameters.

Item	Degrees of freedom	Variance
Between draughtsmen . .	2	$s_B^2$ 0.0175
Within draughtsmen . .	72	$s_W^2$ 0.0180
Between blood films .	12	$s_b^2$ 0.0510
Within blood films .	60	$s_w^2$ 0.0114
Total . . .	74	$s_t^2$ 0.0180

Variance ratio:

$$\frac{s_b^2}{s_w^2} : 4.47^{***} \quad \frac{s_B^2}{s_W^2} : 2.91$$

TABLE 6.  
Analysis of variance of data in table 4.  
Variance of standard deviations.

Item	Degrees of freedom	Variance
Between draughtsmen . .	2	$s_B^2$ 0.0557
Within draughtsmen . .	72	$s_W^2$ 0.0016
Between blood films .	12	$s_b^2$ 0.0013
Within blood films .	60	$s_w^2$ 0.0016
Total . . .	74	$s_t^2$ 0.0030

Variance ratio:

$$\frac{s_w^2}{s_b^2} : 1.23 \quad \frac{s_B^2}{s_W^2} : 34.81^{***}$$

Experiment no. 2 shows another interesting fact: If one compares, in the analysis of the mean diameters, the variance of the grand total in experiment nos. 1 and 2, one finds that this is greater in experiment no. 2, (0.018 against 0.013). Similarly,  $s_w^2$  is larger

in experiment no. 2 than in experiment no. 1 (0.0114 against 0.0078).  $s_w^2$  is a measure for the variation which is due to «error» in the experiment. When this figure is much greater in experiment no. 2 than in experiment no. 1, this means that the assistant in experiment no. 2 works more inaccurately than the assistants in experiment no. 1. The difference between the individual assistants' determination of the mean diameter in experiment no. 1, which has disappeared in experiment no. 2, may therefore be hidden in the higher value for  $s_w^2$ , and experiment no. 2 can therefore not be used as an argument to prove that a difference in drawing does not play a role.

### *Errors due to drawing.*

It is shown in experiment no. 1 that there is a difference between the individual assistants' determination of MD. This difference may be due either to the individual assistants unconsciously choosing large or small blood cells for the drawing (YULE & KENDALL p. 337) or to their drawing the same cells in different ways.

*Experiment no. 3:* A grid-net with approximately  $\frac{1}{2}$  mm distance between the lines was drawn in on a blood film. There were then a little more than 200 blood cells in each square. 5 squares in different parts of the preparation were marked, and assistants A,

TABLE 7.

*The same 5 series of 200 cells in one film drawn by assistant A, B and C.*

*All measurements by assistant E.*

*Mean diameters and standard-deviations in  $\mu$ .*

Series no.	Drawn by assistant		
	A	B	C
1	7.56 - 0.56	7.24 - 0.69	7.61 - 0.73
2	7.69 - 0.58	7.09 - 0.68	7.43 - 0.60
3	7.70 - 0.55	7.22 - 0.66	7.37 - 0.66
4	7.46 - 0.50	7.17 - 0.66	7.62 - 0.66
5	7.56 - 0.50	7.14 - 0.65	7.66 - 0.65



B, and C drew the blood cells in these squares. By comparing the drawings afterwards it was possible to identify the individual blood cells, and thereby make certain that the identical cells had been drawn. All the drawings were measured by assistant E (Table 7). The design of the experiment made the drawing more difficult than usual, a fact which is expressed by the standard deviation being greater in this experiment than in the previous one.

The analysis of variance (table 8) shows that in spite of the fact that in this experiment the same blood cells have

TABLE 8.

*Analysis of variance of data in table 7. Variance of mean diameters.*

Item	Degrees of freedom	Variance	Variance ratio
Between draughtsmen . . . . .	2	0.2671	23.47***
Within draughtsmen . . . . .	12	0.0114	
Total . . . . .	14	0.0479	

been drawn, the variation between the individual assistants is very great. This and similar experiments shows that the drawing of the blood cells must be subject to such large personal errors that this part of the method should be cancelled. Further experiments which were made before the method was given up showed that several conditions were of importance: The intensity of the light in the microscope and on the drawing paper, the height and slope of the working table, whether a plane or concave mirror was used. But, above all, the assistants' degree of fatigue is of importance. Drawings prepared by the end of the working hours were considerably worse than those drawn when the assistant was rested.

### Photography.

If drawing is rejected as a method for reproduction of the blood cells, and if one wants to retain the principle of the projection method, one can photograph the blood cells with a known degree of enlargement and measure the diameter on the photographs.

But with photography a series of new possible sources of error is introduced. The most important is that the field of vision in the microscope is not a plane surface. If, therefore, the centre of the field of vision is focussed, the peripheral parts of the field will be out of focus and therefore blurred.

### *The optical equipment.*

*Experiment no. 4:* A blood film was photographed with different microscopical equipment. 500 blood cells were measured in each of the field-of-vision's central, middle, and peripheral third part. If the curving of the field is of no importance there should be no disagreement between these measurements. (Table 9, 10).

TABLE 9.

*Photography of the red blood cells.*

*Showing the influence of different optical equipment and of the curving of the visual field on MD and s. (values in  $\mu$ y).*

Part of field	Eyepiece		
	Huygen 10 <sub>x</sub>	Periplan 10 <sub>x</sub>	Periplan 10 <sub>x</sub>
	Oil immersion objective		
	achromatic	achromatic	apochromatic
Central part . . . .	7.19/0.51	6.82/0.51	7.16/0.40
Middle part . . . .	6.77/0.60	6.52/0.65	6.91/0.48
Outer part . . . .	6.46/0.62	6.31/0.66	6.54/0.45

TABLE 10.

*Analysis of variance of data in table 9.*

*Variance of mean diameters.*

Item	Degrees of freedom	Variance	Variance ratio
Between different optical outfits	2	0.0862	1.15
Within optical outfits . . . . .	6	0.0991	
Between different part of fields	2	0.2888	9.12*
Within part of fields . . . . .	6	0.0316	

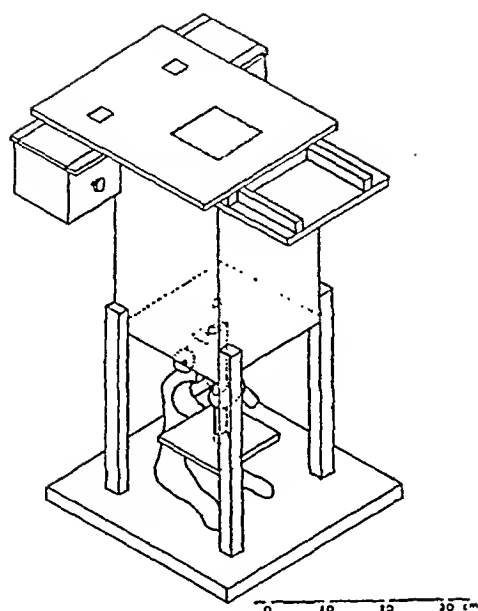


Fig. 3: The authors camera for photography of red blood cells.

The source of light, a 25w,6a pointlight bulb, is placed in a lamp holder in the position of the microscope mirror. The lenses in front of the lamp are adjusted so that the point of image of the light source is positioned in the diaphragm opening of the substage condenser. The source of light is centered once and for all.

The microscope was equipped with a Leitz oil-immersion-objective  $\frac{1}{12}$  achrom. and Leitz periplan ocular 10 x. As film, ordinary electrocardiographic paper was used. This paper has such a narrow margin for exposure that errors in exposure time are eliminated. If the pictures are wrongly exposed they are so poor that they have to be discarded.

The size of the picture, 7 x 7 cm, or approximately 30 % of the whole field of view, was sufficiently small to prevent that the conditions mentioned in experiment no. 4 could be of any importance.

With given optics and tube length, the degree of enlargement depends upon the distance from the ocular of the microscope to the focussing screen. The box was therefore at first, during the construction, only attached to the legs with loose screws and the height was regulated until the degree of enlargement was exactly 1000 x. In this position the box was screwed firmly together.

### *Comparison between drawing and photography.*

*Experiment no. 5:* Two fields with altogether 88 blood-cells, were marked in on a blood film. These 88 blood cells were then drawn and photographed 5 times, and measured by

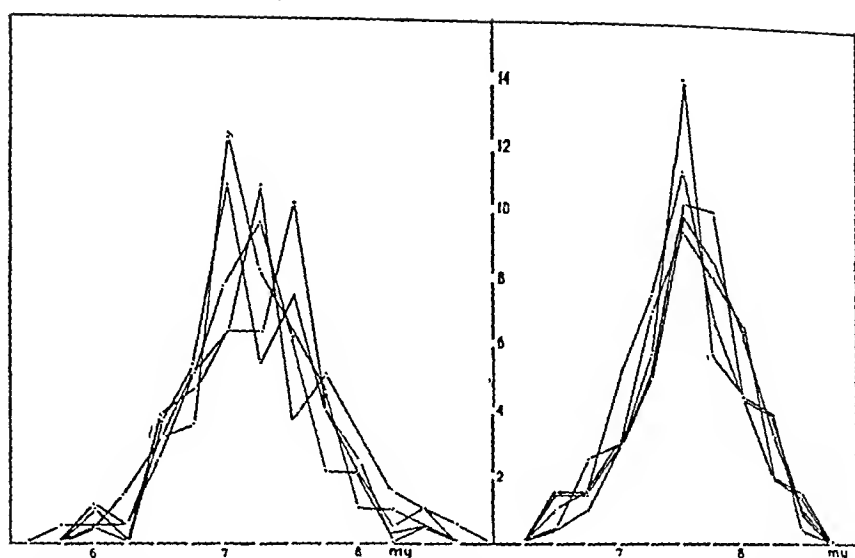


Fig. 4: Frequency curves obtained when the same 88 blood cells were drawn (left) and photographed (right) five times. (Measurements by assistant A.).

assistant A. Fig. 4 shows that the frequency curves are more even and show a better concordance with the photographic method.

*Experiment no. 6:* How great is the improvement in the method when the blood cells are photographed instead of drawn? In order to answer this question the same 5 preparations which were used in experiment nos. 1 and 2 were photographed and measured by assistant A. (Table 11 A.)

The table shows a considerable improvement compared with the results in table 1-A. To ensure that this was not just due to chance, 3 new series, each of 5 preparations, were prepared from normal persons, and these series were photographed and measured.

The first thing one notices by comparing the figures in table 11-A with the corresponding figures in table 1-A is that the diameter of the blood cells has increased. The mean diameter for each blood film is given in table 12.

The cause of this will be discussed later (experiment 13 pg. 68). The second thing one notices, is, that the concordance between the values obtained is much better when the blood cells are photographed (tables 13—14).

TABLE 11.

*Blood films prepared in rapid succession.  
Mean diameters and standard deviations determined by  
measurements on photographs.  
5 series of 100 cells in each film.*

I	Subjects:		IV
	II	III	
A	Assistants:		E
	B	E	
7.76 - 0.48	7.99 - 0.43	7.76 - 0.43	8.23 - 0.47
7.76 - 0.50	8.03 - 0.51	7.90 - 0.46	8.19 - 0.45
7.80 - 0.47	8.04 - 0.46	7.68 - 0.49	8.23 - 0.42
7.77 - 0.52	8.02 - 0.46	7.71 - 0.44	8.20 - 0.46
7.73 - 0.41	8.05 - 0.46	7.62 - 0.47	8.28 - 0.46
7.81 - 0.45	8.00 - 0.39	7.76 - 0.41	8.12 - 0.45
7.76 - 0.47	7.96 - 0.45	7.85 - 0.43	8.19 - 0.42
7.73 - 0.50	8.07 - 0.48	7.77 - 0.47	8.21 - 0.46
7.75 - 0.43	8.06 - 0.44	7.80 - 0.46	8.20 - 0.42
7.79 - 0.45	8.08 - 0.53	7.77 - 0.46	8.18 - 0.48
7.81 - 0.39	8.04 - 0.48	7.78 - 0.55	8.24 - 0.45
7.77 - 0.44	8.07 - 0.50	7.69 - 0.40	8.22 - 0.48
7.77 - 0.50	8.03 - 0.40	7.74 - 0.50	8.24 - 0.51
7.77 - 0.44	8.03 - 0.46	7.78 - 0.45	8.20 - 0.42
7.79 - 0.45	8.08 - 0.46	7.69 - 0.45	8.28 - 0.48
7.76 - 0.41	8.05 - 0.54	7.82 - 0.41	8.17 - 0.51
7.74 - 0.43	8.13 - 0.45	7.83 - 0.42	8.19 - 0.41
7.75 - 0.43	8.04 - 0.45	7.83 - 0.43	8.18 - 0.43
7.72 - 0.44	8.02 - 0.43	7.83 - 0.47	8.18 - 0.47
7.78 - 0.50	8.01 - 0.43	7.88 - 0.48	8.23 - 0.51
7.73 - 0.44	8.11 - 0.59	7.77 - 0.44	8.14 - 0.44
7.74 - 0.48	8.06 - 0.47	7.79 - 0.50	8.10 - 0.40
7.73 - 0.48	8.00 - 0.47	7.80 - 0.44	8.17 - 0.44
7.67 - 0.47	7.99 - 0.48	7.77 - 0.40	8.16 - 0.48
7.60 - 0.44	8.04 - 0.50	7.76 - 0.41	8.18 - 0.41

The variance ratio shows that there is now no significant difference in the determination of the standard deviation in the different preparations when the determination is made by the same assistant. There is still a significant difference in the mean diameter between the single preparations in series I and IV and a probable difference in series III. Series II has no significant difference. This is due to the fact that this assistant's  $s_p^2$  is considerably smaller than that of the other assistants. The reason is that this assistant

TABLE 12.

*Showing the difference in MD when blood cells are measured by the same assistant according to Price Jones' method and on photographs.*  
*(Data from table 1—A and 11—A. Values in my).*

Blood film no.	Drawings	Photographs	Difference
1	7.00	7.76	0.76
2	7.03	7.77	0.74
3	6.92	7.78	0.86
4	7.17	7.75	0.58
5	7.09	7.69	0.60
Average	7.04	7.75	0.71

TABLE 13.

*Analysis of variance of data in table 11.*  
*Variance of mean diameters.*

Subjects	Variance			Variance ratio
	Total	Between blood films	Within blood films	
I	0.0020	0.0059	0.0012	4.92**
II	0.0015	0.0005	0.0017	3.40
III	0.0041	0.0093	0.0031	3.00*
IV	0.0018	0.0061	0.0010	6.10**

TABLE 14.

*Analysis of variance of data in table 11.*  
*Variance of standard deviations.*

Subjects	Variance			Variance ratio
	Total	Between blood films	Within blood films	
I	0.0011	0.0010	0.0011	1.10
II	0.0019	0.0017	0.0019	1.12
III	0.0013	0.0009	0.0014	1.55
IV	0.0011	0.0010	0.0011	1.10

has a personal methodical error in the measurements. This assistant «draws towards the center» and all her measurements are therefore gathered around the figure 8 my. This error is discussed further on page 62.  $s_w^2$  is a measure for the causes of variation which are not due to differences between the preparations. While  $s_w^2$  in experiment no. 1 was 0.0078, in experiment no. 2, 0.0114 and in experiment no. 3, 0.0114, we find in experiment no. 6 where the blood cells have been photographed, that the figure has decreased to between 0.0010 and 0.0031 with a mean of 0.00175. This shows that important errors have been eliminated.

### Other technical errors.

#### *The spreading of the blood film.*

PINEL has shown that the distribution of the leucocytes in a blood film depends upon certain factors such as the slope of, and the pressure applied on, the cover glass, the speed with which the blood film is spread, and other factors. In order to eliminate these errors he has constructed a «hémocétaleur à commande hydraulique».

*Experiment no. 7:* According to PINEL's description I made a small apparatus for mechanical preparation of the blood film:

A plane object slide, carefully cleaned, is placed in a frame on a small apparatus table which is exactly horizontal. A small sledge with a plane ground cover glass at an angle of  $45^\circ$ , guides the cover glass across the slide. A falling weight provides the power for the movement and a brake regulates the speed of the sledge. A weight of approximately 60 grams presses the cover glass against the slide (for further reference see PINEL).

After the apparatus was adjusted, 5 mm<sup>3</sup> blood were placed on the slide in such a way that the blood drop spread out along the edge of the cover glass. The weight pulled the cover glass across the slide and the blood film was thus prepared completely mechanically. The blood films were apparently quite perfect. The length of the blood film varied between 54 mm and 56 mm. The width was 20 mm, and the area of the blood films varied therefore between 1080 mm<sup>2</sup> and 1120 mm<sup>2</sup>. The blood

cells were evenly distributed and did not, on ordinary inspection, appear to be deformed. Two series, each of 5 preparations, were made by this method, stained, photographed and measured, as in experiment no. 6 (tables 15, 16).

TABLE 15.

*Blood films prepared with «hémocytateur à commande hydraulique».*

*Measurements from photographs by assistant E.*

*Mean diameters and standard deviations (in  $\mu$ ) in series of 100 blood cells.*

Blood film	Subject	
	V	VI
no. 1	7.82 - 0.43	7.53 - 0.39
	7.91 - 0.48	7.48 - 0.45
	7.88 - 0.49	7.45 - 0.51
	7.92 - 0.51	7.53 - 0.48
	7.86 - 0.51	7.56 - 0.54
no. 2	7.76 - 0.48	7.41 - 0.43
	7.92 - 0.47	7.41 - 0.40
	8.00 - 0.46	7.36 - 0.30
	7.97 - 0.50	7.63 - 0.46
	7.86 - 0.48	7.58 - 0.51
no. 3	7.84 - 0.44	7.72 - 0.54
	8.00 - 0.46	7.68 - 0.46
	7.82 - 0.47	7.56 - 0.40
	7.88 - 0.49	7.55 - 0.52
	7.92 - 0.48	7.75 - 0.44
no. 4	7.58 - 0.53	7.72 - 0.47
	7.63 - 0.49	7.69 - 0.43
	7.51 - 0.50	7.64 - 0.53
	7.45 - 0.51	7.57 - 0.48
	7.51 - 0.54	7.52 - 0.54
no. 5	7.67 - 0.44	7.48 - 0.49
	7.74 - 0.45	7.42 - 0.49
	7.70 - 0.55	7.44 - 0.38
	7.67 - 0.47	7.56 - 0.51
	7.67 - 0.47	7.39 - 0.43

In spite of the fact that the films here are prepared in a completely mechanical way there is still a definite difference between the preparations.  $s_w^2$  has as well increased compared with the corresponding figures in the previous experiments. Since the



TABLE 16.  
Analysis of variance of data in table 15.  
Variance of mean diameters.

Subjects	Variance			Variance ratio
	Total	Between blood films	Within blood films	
V	0.0254	0.1308	0.0042	31.14***
VI	0.0125	0.0395	0.0071	5.56**

technique for spreading the blood film is the only thing which has been changed, one may assume that the mechanical preparation of the blood film does not represent any advantage in the measurement of the blood cells' diameter. The experiment does not show that the spreading of the film is without importance, but the considerable difference between the preparations found in this experiment is surprising, considering the uniform size and faultless appearance of the preparations.

*Does the spreading alter the cell-size?* As mentioned in the introduction, are the opinions on this question divergent. To settle the question, the author made several comparative measurements, without being able to prove any definite difference between the dry and the wet method. But I am aware that such parallel investigations are of conditional value. I therefore tried to settle the question by the following experiment:

*Experiment no. 8:* In experiment no. 7 where the blood films were prepared with «hémocytaleur», it was found that 5  $\text{cm}^3$  blood covered an area of 1080—1120  $\text{mm}^2$ . In the moment of preparation the thickness of the blood layer is therefore approximately 5  $\mu$ . The blood cells have an average thickness of approximately 2  $\mu$ , and one may therefore presume that they are completely submerged in plasma at the moment of preparation. The total area of the blood film does not decrease during the drying. If, therefore, the blood cells change in size during drying, the distance between the individual blood cells must change.

One blood film was prepared with «hémocétaleur» and a part of the film was immediately covered with a cover glass greased along the edge. The blood under the cover glass remained fluid whereas the rest of the preparation dried up. Another coverglass was placed over the dry part of the preparation, and both parts were photographed. Two equally large photographs from these two fields were then selected, and all blood cells in the pictures were cut out. By weighing the blood cells which had been cut out, and the rest of the photograph, one obtained a measure for the part of the field which was covered by blood cells, and how great a part of the field which was «space between». There was no measurable difference of the «space between» in the two photographs, and I therefore agree with those authors who state that the diameter of the red blood cells remains unaltered during the act of drying.

The authors who have another opinion (jfr. pg. 14) may have overlooked some points: COLLATZ states that the blood cells increases in diameter when blood films are made. But he measured the dried cells with an ocular micrometer, whereas the wet cells were photographed. These two methods certainly do not give concordant results.

PRICE JONES (2), who states that the cells decrease in size during drying seems to have used oil immersion objective for the investigation of the blood film, and dry lens objective when measuring the wet preparations. In experiment no. 6 it is shown that these two methods are not equal. Use of different coloured light and the colour of the film, may also come in as possible factors explaining the differences observed by some authors.

PONDER (1) objects that in Price Jones' method the blood cells are deformed when the blood film is prepared so that they are not of the same size in the different parts of the preparation. And v. BOROS (1) objects that in the measurement of the blood-cells' diameter in blood films, their diameter depends upon the thickness of the preparation. It is an unalterable demand in Price Jones' method that the blood film shall be so thin that the single blood cells are completely isolated from each other, and VON BOROS' objection needs therefore not be considered. PONDER's objection can be estimated by experiment no. 6. In each series, 100 blood cells are measured, and the standard deviation in all

the series averages 0.45 my. The standard error of MD is therefore  $\frac{s}{\sqrt{n}} = \frac{0.45}{\sqrt{100}} = 0.045$ , and the square of the standard error is 0.002025.  $s_w^2$  must be allowed to have approximately this value and table no. 13, pg. 48 show that it is only exceeded in series III. The individual fields in this experiment were placed, one field in each corner and one in the centre of the preparations. They have therefore been selected from different locations in the preparation, and there is nothing to substantiate that PONDER's objection applies here. Neither have I been able to find a difference as mentioned by Ponder in a series of similar experiments which I have made. It will later be shown (experiment no. 16), that deformation of the blood cells as mentioned by PONDER, really does occur. But by using Price Jones' technique, measuring the largest and the smallest diameter of each cell and then taking the mean of these two figures, this source of error is eliminated.

*The staining of the blood film.*

If the colour of the blood cells is of any importance one would obtain a different diameter when the same blood cells are photographed in a different coloured light.

*Experiment no. 9:* Two fields in a blood film, with altogether 98 red blood cells, were photographed 5 times in white light, and 5 times with different coloured light (table 17). The variance ratio between white light/coloured light is 440\*\*.

TABLE 17.

*Mean diameters (in my) of the same 98 blood cells when photographed in white and in coloured light.*

White light 7.77	Red light	7.66
7.74	Violet light	7.68
7.67	Yellow light	7.67
7.70	Yellow-green light	7.71
7.71	Blue light	7.86

The difference between the lowest and the highest MD when the same blood cells are photographed in different light is 0.20 my or 4.5 times the standard error.

*Experiment no. 10:* It is possible that the colour is of importance as shown by experiment no. 9, but then the difference must be due to the staining of the preparations, because all photography in the previous experiments was made with the same source of light. It is known that the colour of the blood films depends upon the Ph during the staining. 10 blood films, made in rapid succession from the same person, were mixed so that their relative sequence was unknown. They were divided into two series, A and B. One preparation from each series was stained at different Ph. The diameter was measured after photography in white light. 200 blood cells were measured in each preparation. In this experiment it is therefore not the same cells which are measured. By mixing the preparations it was intended to eliminate the possibility that the blood cells might have different sizes in the individual drops.

The preparations which were stained at Ph 5.6 had a light rose stain, preparations which were coloured at Ph 8.2 were dark blue. In this experiment (table 18) there is a steady increase in MD from the rosa to the blue preparation. The difference is respectively 0.32 and 0.27 my for the two series. The variance

TABLE 18.

*MD (in my) of blood films from the same person when stained at different Ph, and photographed in white and in yellow-green light (N: 200).*

	White light		Yellow-green light	
	Series A	Series B	Series A	Series B
Ph 5.6	7.52	7.56	7.65	7.67
Ph 6.3	7.59	7.65	7.63	7.65
Ph 6.8	7.69	7.65	7.65	7.67
Ph 7.7	7.66	7.76	7.64	7.66
Ph 8.2	7.84	7.83	7.62	7.69

ratio (between Ph/within Ph) is 14.32\*\*. In this experiment, as in the previous one, the MD of the blue preparation deviates most from the others.

If it is the different colour which is the cause of the difference in MD, this difference ought to disappear when the preparations are photographed in monochromatic light. They were therefore photographed once more, this time in a strong yellow-green light (The light was not purely monochromatic).

The variance ratio between Ph/within Ph now is 6.08 which is not significant. The difference in MD between the individual preparations is gone.

The objection may be raised that the colour of the preparations may result in different values for the blood cells' MD in experiments like the two just mentioned, but that this cannot have any practical significance since the staining of the preparations always is done at the same Ph and since the photography always is done with the same source of light. That this reasoning is not correct is shown by the following experiment:

*Experiment no. 11:* In order to decide if the diameter of the blood cells changes in the same person during any length of time, films from 5 persons who were all normal, were taken over a certain period. In the case of 4 of these persons, the preparations were stored unstained in a drawer until, by the end of the experiment, they were all stained and investigated. In the case of the 5th person, the preparations were by chance stained as they were prepared. Mean diameters and standard deviation in this series when the preparations were photographed in white light and in yellow-green light are given in table 19.

By calculating the mean value of MD and the distribution around this mean for each series in the two experiments, the figures in table 20 are obtained.

The table shows that there is a considerable variation in MD in the same person when the photography is done in white light, but the variations decreases in all cases when the photography is done in yellow-green light. The variation is decidedly smallest between preparations from T. S., whose preparations were stained at the same time as they were taken.

The chemical processes which take place in a blood film stained

with the usual polychromatic stains are little known. But it is known that a uniform stain is not always obtained even if all technical details like staining time, concentration of staining

TABLE 19.

*MD and s (in my) in blood films from five normal subjects, taken during 8 weeks and photographed in white light and in yellow light (N: 200).*

Date	Blood films from:				
	S. B.	A. J.	E. P.	S. S.	T. S.
<i>Photographed in white light:</i>					
11/4	7.73 - 0.50	7.45 - 0.43	7.53 - 0.43	7.28 - 0.47	7.54 - 0.44
20/4	7.58 - 0.44	7.73 - 0.48	7.99 - 0.47	7.43 - 0.42	7.35 - 0.49
30/4	7.28 - 0.47	6.86 - 0.52	7.47 - 0.41	6.99 - 0.43	7.34 - 0.43
12/5	7.54 - 0.44	7.43 - 0.41	7.79 - 0.43		7.49 - 0.47
4/6					7.47 - 0.44
<i>Photographed in yellow light:</i>					
11/4	7.62 - 0.45	7.45 - 0.45	7.53 - 0.43	7.29 - 0.42	7.45 - 0.44
20/4	7.56 - 0.44	7.62 - 0.41	7.69 - 0.43	7.31 - 0.47	7.43 - 0.41
30/4	7.68 - 0.47	7.51 - 0.42	7.48 - 0.42	7.12 - 0.43	7.37 - 0.47
12/5	7.45 - 0.43	7.59 - 0.42	7.41 - 0.44		7.49 - 0.45
4/6					7.47 - 0.44

TABLE 20.

*Average values of MD (in my) from table 19.*

Subject	Photographed in	
	White light	Yellow light
S. B.	7.53 $\pm$ 0.19	7.58 $\pm$ 0.10
A. J.	7.37 $\pm$ 0.37	7.54 $\pm$ 0.08
E. P.	7.70 $\pm$ 0.19	7.53 $\pm$ 0.04
S. S.	7.23 $\pm$ 0.22	7.21 $\pm$ 0.10
T. S.	7.44 $\pm$ 0.08	7.44 $\pm$ 0.04

fluid, constant Ph, etc. are adhered to. In all cases the blood cells themselves come in as an unknown factor. Polychromasia has thus, for a long time, been regarded as a sign of the presence of young blood cells. A lesser known, but in this connection a very important point, is that the blood cells' reaction to the staining fluids changes when they are stored unstained for some time. Such old blood films will, by usual staining, become more bluish than freshly prepared blood films, and experiments 9 and 10 show that they will then give a higher value for MD. This might be the explanation when MOGENSEN (1 p. 29) states that *«the act of preparation of the film has the same effect on each blood cell of the film, they are equally «enlarged», or «diminished».*

In order to avoid this source of error one might consider staining the blood cells by another technique as is done by GÜNTHER. He stains the blood cells black with osmic acid. Or the blood cells may be photographed unstained. But this did not give satisfactory results with my apparatus. Finally, one may utilize the method which I have chosen, namely, to photograph the cells in yellow, nearly monochromatic light, and thereby exclude the red and blue rays of light from the photographic plate.

#### *Diurnal variation. Variation after exercise.*

This has been mentioned by several authors, particularly PRICE JONES (2) and WIECHMANN & SCHÜRMEYER (2). These authors report a diurnal variation in MD between 0.3 and 0.6 my, and a similar variation after exercise. Both authors are of the opinion that the change is due to increased CO<sub>2</sub> content of the blood. The phenomenon has, on the other hand, been denied by several others (PONDER & MILLAR, DRUYERRE, MILLAR & PONDER).

The author has, in a series of experiments, not been able to prove any definite diurnal variation in healthy or sick persons. Neither was any definite difference found after moderate exercise. But in an experiment where the author measured his own blood cells before and after a quick walk of 14 kilometers a difference of 0.32 my was found when the blood films were photographed in white light (7.74 and 8.06 my). When the same preparations were re-photographed in yellow light the difference disappeared.

(MD 7.76 and 7.78 my). I presume therefore that the difference in this experiment was caused by the fact that the two blood films stained differently and that the one which was taken after exercise was more blue coloured than the first. I regard this as the possible cause of the difference which the previously mentioned authors reported. The difference which WIECHMANN & SCHÜRMEYER have found in venous and arterial blood and the difference which some reports in patients with acidosis may possibly have the same cause.

### The relative importance of the different errors.

The experiments here reported show that PRICE JONES original method ought to be replaced by photography. But even when photography is used, the MD is determined differently in the individual blood films, as shown in experiment no. 6. It may be calculated (BONNIER & TEDIN, pg. 116) that the part of the total variance which is due to differences between the blood films is 44 % in series I, 28 % in series III and 51.5 % in series IV of experiment no. 6. This error can no longer be due to the measurement, since all films in each series are measured by the same person. Neither can it be due to the reproduction, since this is done photographically. The error must be due to the preparation or the staining of the films, or represent a real difference between the blood used in the individual films.

The error caused by the staining of the film may be estimated by studying experiments nos. 10 and 11.

As mentioned under experiment no. 8, the standard deviation in normal blood preparations is approximately 0.45 my. In experiments nos. 10 and 11, 200 blood cells were measured in each preparation and the standard error is therefore  $\frac{0.45}{\sqrt{200}} = 0.032$  my.

In experiment no. 10, when the blood cells are photographed in yellow light, the error is 0.02057 my, which is less than the standard error. We have, therefore, in this experiment, no reason to believe that technical errors due to the colour of the light influence the measurements to any great extent. In experiment no. 10, however, all preparations were taken simultaneously from the same incision. Actually it will be a question of



comparing preparations taken over some period of time from the same person, and it is therefore more correct to judge the error on the basis of experiment no. 11 where the films have been prepared in this way. In this experiment as well, 200 blood cells have been measured in each preparation and the error should therefore not exceed 0.032 my. Table 20 shows that when the preparations are photographed in yellow light the error ranges between 0.04 and 0.10 my. It is worth noting that the series which was stained immediately only had an error of 0.04 my.

Several similar experiments shows that one, by careful technique, can bring the variation down to between 1 and  $2.5 \times$  standard-error when the preparations have been taken from normal persons. It seems therefore as if a technical error still remains when the preparations are taken over a longer period of time. I have not been able to explain this source of error. Perhaps the spreading of the film plays a role, perhaps it is caused by conditions in connection with the sampling. Finally it may be possible that the blood cells are subject to real changes during the course of the test period. The method will be used for investigation of pathological blood samples. The error-variance of the method must therefore be determined by investigating pathological, and not normal preparations. This point will therefore be dealt with separately. (Chapter VII.)

In experiment no. 6 it was shown that the blood cells' MD, on an average, were 0.71 my greater when they were photographed than when they were drawn. It was further mentioned in the introduction that other authors have shown that the blood cells appear to be greater when they are investigated with a dry lens objective than when an oil immersion objective is used. Finally, experiments nos. 1 and 3 show that there is a systematic difference between the individual assistants' determination of the mean diameter. In experiment no. 1 pg. 36 the average MD determined by assistant A is 7.04 my, by B, 6.92 my and by C, 7.00 my, and in experiment no. 3 assistant A finds an average MD of 7.59 my, B, 7.17 my and C, 7.53 my. A thus continuously finds the highest values and B the lowest. This difference is not due to any of the previously mentioned sources of error, but is of the same class as the difference between drawing and photography and between an oil immersion and a dry lense objective.

*Experiment no. 12:* In order to explain the phenomenon it is necessary to determine the size of the difference: A grid-net with approximately  $\frac{1}{2}$  mm distance between the lines was drawn in a blood film. Squares containing in all about 600 blood cells were marked out. Assistants A and B, who in the earlier experiments had the greatest difference between their results, then drew 500 of these blood cells both using oil immersion and dry lens objectives. The same blood cells were also photographed with the two optical equipments, and the photographs were measured by the two assistants. The enlargement of the microscope was each time controlled by both assistants, who agreed that the enlargement each time was exactly 1000 x. Table 21 shows the results of the experiment.

TABLE 21.

*The influence of the technique used on the value (in  $\mu$ ) of MD. The same 500 blood cells measured by two assistants with varying technique.*

Technique	Assistant		Difference between A and B
	A	B	
Drawing with oil imm. obj. . .	7.61	7.19	0.42
Drawing with dry lens obj. . .	7.82	7.61	0.21
Photo. with oil imm. obj. . .	8.04	7.76	0.28
Photo. with dry lens obj. . . .	8.21	8.11	0.10

The cause of the considerable difference which appears both between the two assistants as well as between the techniques employed will be discussed in the next chapter.

### Résumé of Chapter III.

Using Price Jones' method for measuring the red blood cells' diameter as a starting point, a review of the technique for measuring the blood cells by projection is given. Experiment no. 2 shows that both the determination of MD and the determination of  $s$  depend upon personal qualities in the individual assistant, connected with the measuring of the blood cells in the drawing. Experi-

ment no. 3 shows that even the drawing of the blood cells is subject to errors, which are so great that this link in the method ought to be cancelled.

Then follows a description of a camera for photography of the blood cells, constructed by the author; and in experiments 5—6 it is shown that important sources of error are eliminated when the blood cells are photographed instead of drawn.

In experiments nos. 7—11 errors connected with the preparation of the blood film, the staining, and the colour of the light are investigated. It is shown that the colour of the light and the stain of the blood films play an important role for the determination of the mean diameter, and that the error can be further reduced when these conditions are standardized.

It is discussed whether the diameter of the blood cells is altered during spreading, drying and staining. The author is of the opinion that no such changes occurs.

Whether diurnal variations and variations after exercise do occur in MD, as mentioned by PRICE JONES and others is investigated. The variation which these authors have shown, is assumed to be due to different staining qualities of the blood cells under different conditions. In experiment no. 12 it is finally shown that the microscopic equipment and personal qualities must play a role in the determination of the red blood cells' diameter.

## CHAPTER IV.

### Measurement by projection. The causes of the errors.

#### Human Bias.

In chapter III it was shown that the projection method has errors partly due to technical details in the method and partly due to human bias. In this chapter it will be shown that the part of the errors which are due to human bias is mostly connected with the actual measuring of the blood cells, while the technical errors are due to refraction and scattering of the light at the cell border.

#### *Leptokurtosis.*

PRICE JONES (7), GÜNTHER (2), MOGENSEN (1) and HERNBERG (1) have shown that with a good technique the diameters of the red blood cells should be distributed normally around their mean when the sample is taken from normal persons. The accuracy of an assistant may therefore be measured by comparing his frequency curve with the corresponding normal curve.

When doing this one finds that a number of assistants «draw towards the centre»:

When one measures drawings or photographs of blood cells it soon becomes evident where approximately the mean lies. Some assistants then have an unconscious tendency to register more frequencies in the central class than really should belong there. This phenomenon is most apparent in the case of assistant B. Fig. 5 a shows this assistant's measurements of the 500 blood cells in preparation No. 4 from table 1. And figure 5 b shows

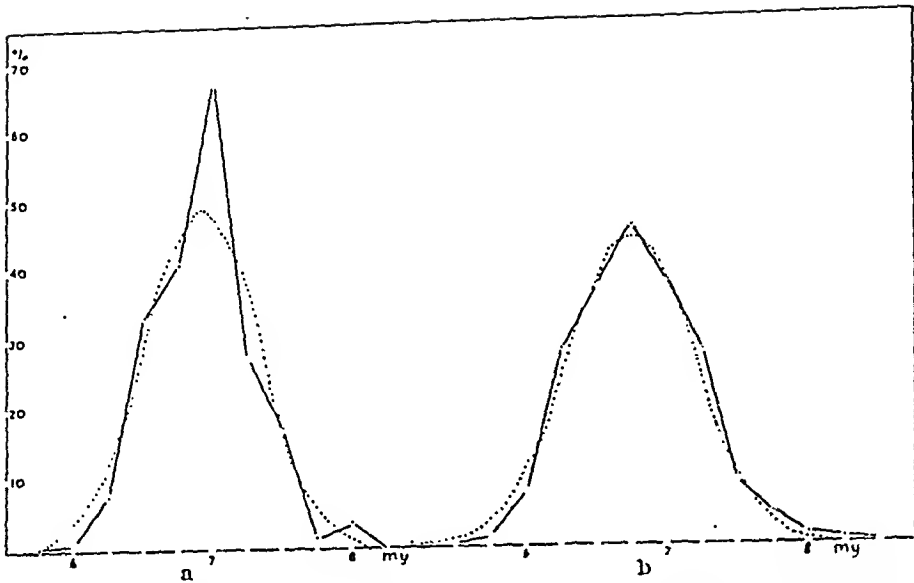


Fig. 5: Frequency curves produced by assistant B (left) and assistant D (right) when measuring the same photographs. Stippled curves: Corresponding normal curves.

the frequency curve of assistant D when he measures the same drawings. The figure clearly shows that assistant B has too many frequencies in the class 7.00 my. Assistant D does not have this error and the curves of this assistant therefore conform very well with the corresponding normal curve. This peculiarity in assistant B leads to the curves being higher and narrower than they should be. The standard deviation of the curves will therefore be small. Table 1 shows that this low standard deviation occurs in all measurements from assistant B. This was a peculiarity in this assistant which was impossible to eliminate even when she was notified of the condition. In other assistants the phenomenon may be less pronounced. It depend partly upon how finely the ruler is graded. A ruler which was graded in 1/1 mm. was used in the beginning of this investigation, and the same phenomenon was then shown by assistant A. The phenomenon was even more pronounced when after the suggestion of ALDER, FREERKSEN and HERNBERG the blood cells were drawn on millimeter paper and the size was determined by counting the squares on the paper. In my hands this method was so inaccurate in the determination of  $s$  that it had to be abandoned. The determination of MD is only slightly influenced by this error.

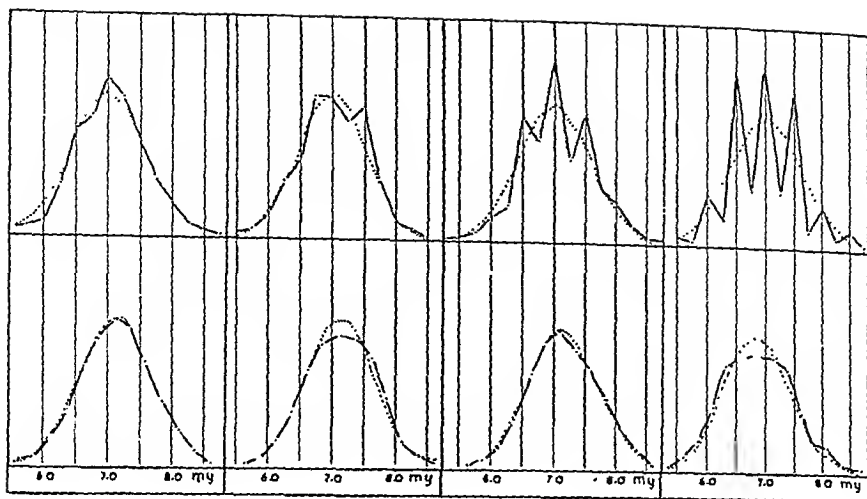


Fig. 6: Frequency curves produced by four different assistants when measuring the same photographs. Upper row: Observed frequencies. Lower row: Adjusted frequencies. Stippled: Corresponding normal curves.

### «Dentated» frequency curves.

Fig. 6 shows another frequently occurring error. In the upper row are given the frequency curves which appear when 4 assistants measure *the same photographs* from a normal blood film. Whereas the first curve shows a good concordance with the corresponding normal curve, the other curves are more and more irregular and dentated. In all curves it is the variate classes ending on .25 or .75 which are subnormal.

This condition is explained by the method used for measuring: The largest and smallest diameter of each blood cell is measured, and the mean of these figures is registered as the diameter of the blood cell. If now both the two measured diameters are whole figures, then the mean must be either a whole figure or a figure that ends on .50. If the mean should end on .25 or .75 one of the measured diameters would have to end on .50. The assistants who obtain subnormal values in the classes .25 and .75 have a tendency to measure the single diameters in whole figures. The whole millimeters are marked with a longer stroke than the halves on the ruler, and these investigators «draw towards» the longest

TABLE 22.  
*Percentage of frequencies in the different variate classes.*

Variate class ending on	Assistant							A
	A	B	C	D	E	F	G	
.00	24.6	31.8	25.7	25.8	26.1	32.6	42.9	31.5
.25	25.2	23.3	25.2	23.9	24.2	18.1	9.2	23.3
.50	24.5	24.7	24.2	26.2	26.0	32.3	32.3	23.9
.75	25.7	23.2	24.9	24.1	23.7	17.0	11.2	21.3
Total number of cells measured	6032	4133	5026	5016	4708	5240	1748	5525

strokes on the ruler. Table 22 shows the distribution by percentage of the frequencies in the four groups of variance classes for a number of assistants and for a large number of observations (1748—6036). The table shows that the phenomenon is not restricted to individual selected curves. Where the error is not very pronounced (A and C) there is an equal number of frequencies in each class, but the assistant who has the error in the most pronounced degree (assistant G), obtains only 9.2 % and 11.2 % of the frequencies in the classes .25 and .75. The last column of the table shows the results of assistant A when he used a ruler which is graded in 1/1 mm. In this case he over-represents the class .00.

*How often does the error occur?* It certainly occurs very frequently. Many of my assistants had the error to a larger or lesser degree, and it is also present in curves published by PRICE JONES, MOGENSEN, VAQUEZ, KATO & KORTUEM, and RODRIGO & BASOS.

This is an error particular for each assistant, and does not disappear even if the scale of the ruler is made finer. YULE thus found, in an experiment where the measurements were made with a ruler graded in 0.1 mm, that one assistant particularly chose the classes .2, .8, .9, and .0, another assistant had 56.9 % of the frequencies in the classes .0 and .5, whereas 43.1 % of the frequencies were distributed among the remaining 8 classes.

POHLE criticised Price Jones' method from another point of view. He mentioned that if all blood cells were circular then the

two measured diameters would be equal, and the mean would therefore always end on either .00 or .50. Such a lack in poikilocytosis does not occur, at least not in a blood film (compare experiment no. 16), but the observed measuring error leads to the same result.

### *Elimination of errors due to human bias.*

Several methods have been suggested. One can either draw the frequency curve and later «smoothe it out» by drawing by hand as suggested by FISHER. But this method may be biased. Or one may add together two and two variate classes so that the width of the class would be 0.50 my as suggested by POHLE and HERNBERG. But then the number of variate classes becomes too small and makes further analysis of the curves difficult. I have therefore chosen the method which has been suggested by MOGENSEN:

He takes the mean of two and two neighbouring classes and refers it to new variate classes with the midpoint between the two. This has been done in figure 6, lower row. The conformity with the normal curve is now satisfactory in all cases except for the last curve where the original errors were so large that the adjustment did not lead to any useful result.

### **Technical errors. Photometry.**

Experiment no. 12 shows that the diameter of the blood cells is determined differently if oil immersion or dry lens objective is used. The size as well varies if one draws or photographs the blood cells and finally the results of the measurements vary with the different assistants. HAMMARSTEN (3) suggests that the cause may be due to refraction phenomena. BOCK & JOMBRES are of the opinion that the cause is due to interference. The condition may be explained if the photographs of the blood cells are examined in the photometer.

In the photometer a ray from a point-light is sent through the photographic plate to a photo-electric cell. This is coupled into a circuit with a sensitive self-registering galvanometer, such as the galvanometer in an electro-cardiograph. The less blackened



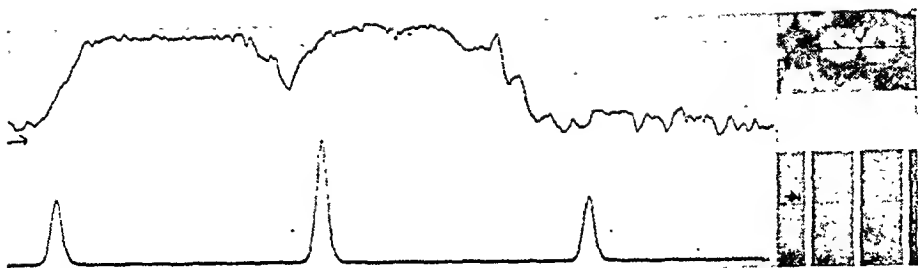


Fig. 7: Right: Photo of blood cells and micrometer scale. Left: Corresponding photometric curves.

the photographic plate is, the more light will reach the photoelectric cell, and the stronger will the galvanometer register. The registered curve of the galvanometer will show to what extent the photographic plate has been blackened.

Fig. 7 shows the results of such an experiment. To the right is shown the photograph of two blood cells and below the photograph of the micrometer scale. To the left are the corresponding photometer curves which are obtained when the light ray from the photometer passes over the picture along the line drawn in the direction from left to right. Figure 8 shows to the right a

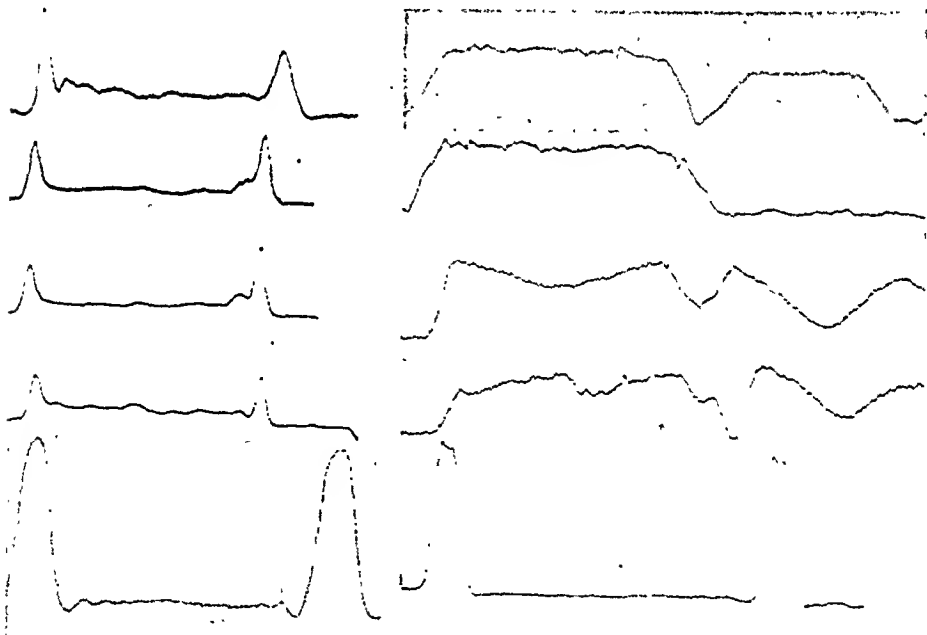


Fig. 8: Photometric curves of red blood cells. Left: Cells photographed with dry lense objective. Right: Cells photographed with oil immersion objective.  
Bottom row: Micrometer scale.

series of such curves from photographs of blood cells investigated with oil-immersion-objective, whereas the curves to the left are from photographs taken with dry lens objective. The bottom curve in each field represents the micrometer scale photographed with the same optical equipment.

These curves show that the transition from the space between the blood cells, which on the photographic plate is black, to the central part of the blood cell which is light, is not sharp, but develops gradually, in the case of the oil-immersion-objective over an area of approximately 1 my. Since the curves from photographs taken with oil-immersion and dry lens objective are so different in appearance, it is unlikely that the transition is gradual. It is more reasonable to assume that the phenomena are caused by refraction and scattering of the light by the blood cell borders.

#### *Errors due to different optical equipment.*

*Experiment no. 13:* In experiment no. 12, the same 500 blood cells were photographed with oil immersion and with dry lens objective, and the cells were measured by assistants A and B. Using oil immersion objective, assistant A found  $MD = 8.04$  my. On these sets of photographs a blood cell which measured exactly 8 my (or 8 mm) was selected. The pictures of this cell photographed with oil immersion and with dry lens objective were then examined in the photometer. The degree of enlargement was both times exactly 1000 x, controlled by photographing the micrometer scale. The two photometric curves of this single cell were then placed beside each other on the same base line. The photometer curve of the micrometer scale was placed underneath (fig. 9). MOXNES has shown that the appearance of the photometer curves depends upon the «veiling» of the plate, i. e. the density in the parts which should have been white. In order to eliminate this error in the experiment, a series of pictures was taken, and two with the same degree of veiling were chosen. The distance between the tops in the micrometer scale (the line m-n) is 10 my. The diameter of the blood cell which was photographed with oil immersion objective should be 8.04 my. By using the line m-n as a scale, this distance can be marked in on the curve to the left. In the photometer curve in line 1. In experiment no. 12 one found further-

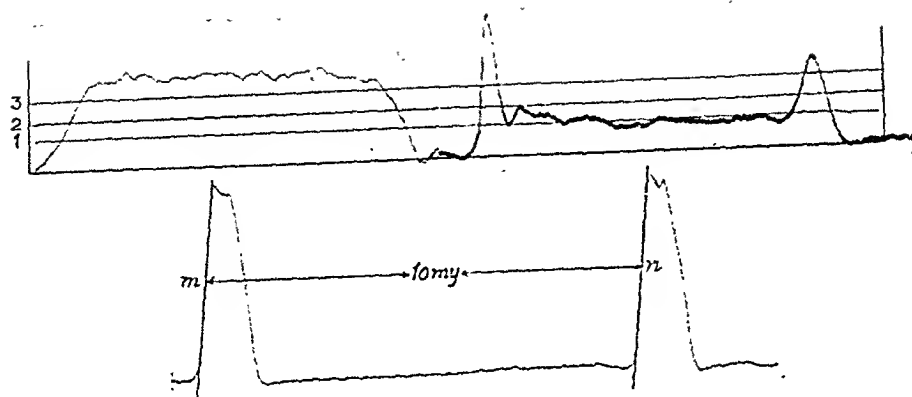


Fig. 9: Photometric curve of the same blood cell photographed with oil immersion objective (left) and dry lens objective (right). Bottom: Micrometer scale. Enlargement 1000  $\times$ . (For further explanation: see text p. 68).

more, that assistant A, by drawing, determined MD to be 7.61 my and assistant B by drawing, determined MD to be 7.19 my. These two diameters as well were marked in on the photometer curve to the left and cut the lines 2 and 3. The lines 1, 2, and 3 are then lengthened until they cut the photometer curve to the right, and the length of the lines 1, 2 and 3 on this curve are measured by means of the scale m-n. These values are compared with the corresponding values from experiment no. 12 (table 23).

The difference between the individual diameters which were found in experiment no. 12 may be explained by assuming that each method and each assistant has his level-line in the photometer-curve. The distance between the level-lines and the base-line

TABLE 23.

*Comparison between MD as determined with dry lens objective (table 21) and diameters measured on the photometric curve.*

	Diameter	
	as measured on the photo- metric curve	as determined in experiment no. 12
Assistant A/Photography .	8.2 my	8.21 my
Assistant A/Drawings . . .	7.8 my	7.82 my
Assistant B/Drawings . . .	7.7 my	7.61 my

gives an expression of the contrast between the light and dark parts of the photograph. The difference which was found in experiment no. 12 may therefore be due to different demands in the contrast between the picture of the blood cell and the surroundings required by the different investigators. The photograph has the smallest demand for contrast and the level-line of the photograph is therefore closest to the base line. Assistant B has the largest demand for contrast, and her level-line is farthest away from the base-line. Since the photometer curve is formed like a trapezium, the level-line, and thereby the diameter of the blood cell, will decrease the farther one gets from the base-line. Assistant B will thereby constantly obtain lower values than the other assistants.

Why do all assistants find an identical value for the degree of enlargement of the microscope?

This is explained by fig. 9. The degree of enlargement of the microscope is determined by measuring the distance between two strokes in the micrometer. Since these have a certain thickness, one will always measure from the edge of one stroke to the corresponding edge of the other. This is the distance  $m-n$ , and fig. 9 shows it to be of equal length, irrespective of the distance from the base-line.

### *Errors due to different coloured light.*

*Experiment no. 14:* In experiment no. 9 (pg. 53) the same blood cells were photographed in different coloured light. Two individual blood cells were marked out on all photographs and the pictures of these two cells were examined in the photometer. The photometric curves are reproduced in fig. 10, central field, while the part of the spectrum used in the different exposures is indicated to the left.

In the foregoing experiment it was shown that each assistant has his specific demand for contrast between the blood cells and the surroundings. Each assistant will therefore have a fixed distance from the base-line to his level-line on the photometric curves. As the curves are shaped like a trapezium, the diameter measured at a fixed level will depend upon the distance between the sidelines of the curve, *and on the slope of the sidelines*. If the angles between the individual sidelines of the photometric curves and the base-line are measured, one finds that the curves represen-



Fig. 10: The same two blood cells photographed in coloured light. Left: Part of spectrum used. Middle: Photometric curves. Right: Slope of curves as measured on the original curves.

ting the pictures taken in red light have the smallest angles, whereas those taken in blue light have the largest angles (fig. 10, right). Red light should, according to this, give the lowest values for MD, blue light the highest values, as already observed in experiment no. 9.

#### *Errors due to different Ph in staining fluids.*

The photographs of the blood films which had been stained at different Ph were finally examined. Table 24 shows that there is good concordance between Ph, the observed values for MD and the angles of the corresponding photometer curves.

According to the table one might expect that preparations stained at Ph 8.2 have the highest values for MD, which they indeed have. These preparations had, as previously mentioned, a deep blue colour.

These experiments therefore show that the technical errors which were found in the previous chapter are due respectively to the assistants and the photographic plate's demand for contrast be-

TABLE 24.

*Photometry of blood-films stained at different Ph.*

Ph	Mean Diameter (in my)		Slope of Photometric curve			
	Series A	Series B	Series A		Series B	
5.6	7.52	7.56	73°	75°	74°	75°
6.3	7.59	7.65	75°	75°	77°	78°
6.8	7.69	7.65	78°	77°	79°	78°
7.7	7.66	7.76	79°	79°	82°	83°
8.2	7.84	7.83	81°	81°	83°	83°

tween the blood cell and its surroundings. The fact that an object gives pictures of different size when photographed in different coloured light is mentioned in several text books on photography (MERTÉ and HENNEY & DUDLEY). Furthermore, it is well known that the medium between the object and the lenses plays a role in the size of the photographic picture when the light rays through the medium are not parallel (HENNEY & DUDLEY). And finally, it is well known that individuals with the same visual acuity may have considerably different ability to distinguish objects with little contrast (LUCKIESH & MOSS). It remains to find out if those who investigated the preparations in the previous experiments really had such different contrast sensitivity.

*The assistants' different powers of distinction.*

*Experiment no. 15:* A star figure as shown in fig. 11, was drawn on a cardboard plate. If such a figure is rotated quickly the central part will appear white, the peripheral part black, and the space between will have an even transition from black to white. This can be shown graphically in the form of the trapezium-like figure at the bottom of fig. 11, which has great similarity with the photometric curves in figs. 7 to 10. Five such figures were made, and were painted to resemble as closely as possible the colours seen in a blood film. The figures were then placed on a centrifuge and the assistants measured the central part as the figure rotated. The diameter of the star was 160 mm, the central part 60 mm.

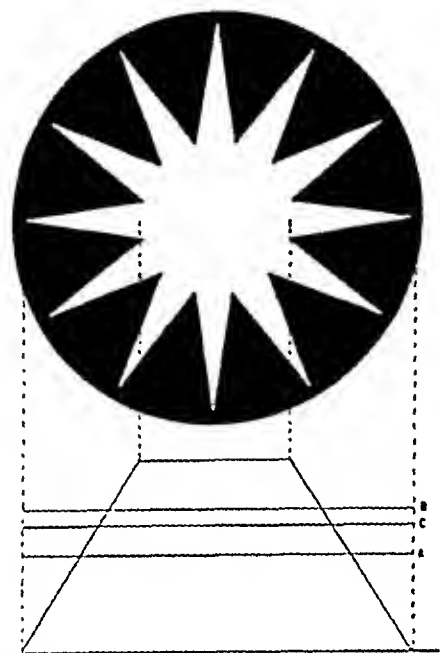


Fig. 11: The assistants different power of distinction.

Assistant A determined the central part to be  $108.9 \pm 3.6$  mm.  
 Assistant B determined the central part to be  $86.2 \pm 5.7$  mm.  
 Assistant C determined the central part to be  $93.5 \pm 12.5$  mm.  
 Each assistant: 25 measurements.

If these level-lines are drawn in on fig. 11, the level-lines for the individual assistants comes in the same order as on the photometric curves. The present investigation was done during the war and one might think that a lack in vitamin A would cause a change in contrast sensitivity. However, no changes occurred after the assistants had taken cod-liver oil in adequate doses. Frequent control measurements and double observations showed that it was a constant condition peculiar to each assistant.

### Importance of the observed errors.

The personal errors are, as shown, of two types. Firstly, the tendency of some individuals to «draw towards the centre». When this quality is present in a pronounced degree, the determination of the standard deviation will be influenced; to a smaller extent

it will influence the determination of the mean diameter. The frequency curves will be leptokurtic and will be of little use for further analysis. If one assistant constantly finds  $s$  lower than 0.42—0.43 my in normal individuals it is very likely that this error occurs.

*Secondly* the tendency to produce «dentated» frequency curves. When this appears in a moderate degree, the determination of MD and  $s$  is not influenced to any important extent and the error may be eliminated by smoothing out the curves in one of the ways which have been described. When the tendency is more pronounced, the assistant can not be used in investigations of this kind.

*The technical errors* do not bring in any deviation in the form or the width of the curves, but the determination of the mean diameter depends very much on the method which is used, and is to a certain extent typical for the method which is employed: Between a dry lens and an oil immersion objective there will be a difference in MD of 0.2—0.4 my when the blood cells are drawn, 0.15—0.35 when they are photographed, provided the measurement is done by the same person. The difference between the measurements of the individual assistants may be of the order 0.4—0.5 my. The total variation which may appear if different assistants utilize different techniques may be as high as 0.9—1.0 my (experiment no. 12). The staining of the blood film and the colour of the light play a certain role. The lowest value of MD is obtained by using red light, the highest value for MD by using blue light. The difference amounts to 0.3 my (experiment nos. 9—10).

### Résumé of Chapter IV.

The errors of the method, which were found in the previous chapter, are caused by two conditions:

1) «*Human bias*». The errors due to this cause are mostly connected with the measurement of the blood cells. The error influences the measurements in two ways:

*Either* by making the observed curves too narrow and too high, leptokurtic, because the assistant «draws towards the centre». When this error is pronounced it will lead to a too low value for the standard deviation.



Or by making the observed curves irregular and dentated (fig. 6). If this error is particularly pronounced the assistant in question is unfit for making measurements. When it is less pronounced it can be eliminated by smoothing out the curves. The degree of error is typical for the individual assistant in the case of both these errors.

2) «*Technical errors*». These errors which chiefly influence the determination of the mean diameter are caused respectively by the individual assistants' and the photographic plate's different demands for contrast between the blood cells and their surroundings. The observed differences in the determination of the mean diameter which are due to different optical equipment, colour of the light, or the stain of the preparation, may be referred to this group of errors, and are explained as results of «refraction and scattering of the light by the blood cell borders». In the last part of the chapter the magnitude of the observed errors is discussed.

## CHAPTER V.

### Other methods of measurement.

It was mentioned in the introduction, that the diameter of the blood cells could be measured by *projection* as described in the two previous chapters, or by *direct micrometry*.

The direct micrometry may be used on blood films or on blood cells suspended in plasma or in another diluting fluid.

#### Direct micrometry in blood films.

One type of ocular micrometer is a glass screen with an arbitrary scale placed in the ocular of the microscope. This scale is adjusted against an object micrometer by regulating the tube length and the optical equipment of the microscope. The use of this type of micrometer gave very uncertain results in my hands. The results are inaccurate, partly because the strokes lie very close together, and partly because interference between the scale and the edge of the blood cell makes an exact reading impossible. All assistants had a tendency to draw towards the centre. Practical experiments showed that these errors had a tendency to be equalized and the mean diameter showed fairly good concordance in repeated measuring. But the determination of  $s$  and the shape of the curves was so uncertain that the method was quickly abandoned. The method is extremely laborious. One is obliged to use light of low intensity in the microscope. And I agree with H. C. GRAM (1) and HORNEFFER that 100 blood cells is the greatest number one can measure before accuracy is jeopardized. The *filar micrometer* was then tried. This is an ocular where a thin

thread is moved across the field of vision by means of a micrometer screw. The movement of the thread is read off on a scale. It is adjusted like the previous one against an object micrometer. With the filar micrometer I was using, one unit of the scale corresponded to 0.013 my by 1000x enlargement; apparently a great degree of accuracy. But when the distance between two of the strokes on the object micrometer (10 my) was measured 500 times the distance varied with  $\pm 4.72$  units. When measuring blood cells with diameter 7.5 my, this corresponds to a variation of  $\pm 0.15$  my which is the real uncertainty in the determination with this apparatus. The error is equalized by making many measurements, so the determination of MD will be fairly good. But the determination of  $s$  and the shape of the frequency curves will be equally as uncertain as with the first micrometer (fig. 12). Interference as well plays a role, and the method was equally strenuous as the previous one. But the deciding argument against the use of direct micrometry in blood films is that the

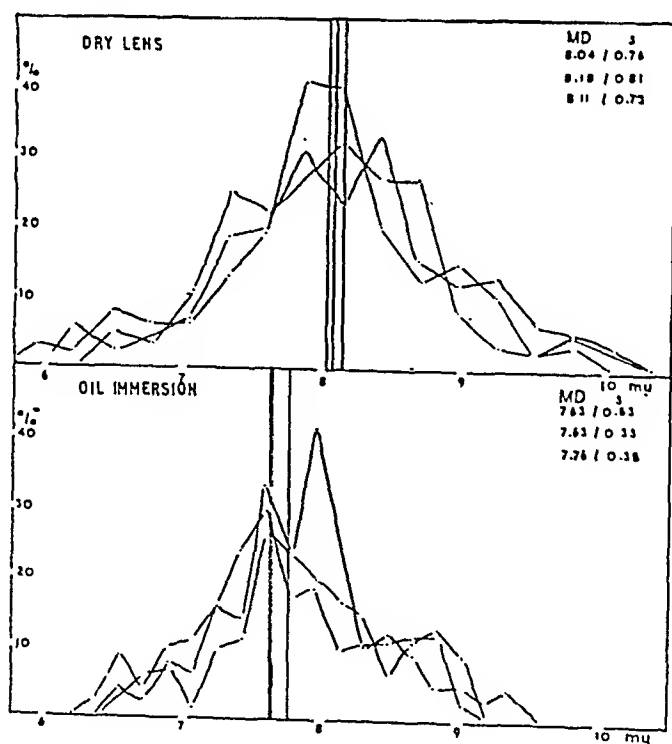


Fig. 12: Frequency curves obtained when measuring the same blood film three times with ocular micrometer ( $N = 200$ ).

blood cells are deformed in the act of preparation. With the micrometer the blood cells are only measured in one direction, usually in the lengthwise direction of the blood film. BOCK & JOMBRES have shown that the diameter of the blood cells here is larger than across the preparation. They find the difference to be 0.24 my.

*Experiment no. 16:* 5 blood films from normal persons were photographed. 200 blood cells in each film were measured both in the lengthwise and the crosswise direction of the slide (table 25).

TABLE 25.

*Difference in MD (in my) when the blood cells are measured in the lengthwise and the crosswise direction of the slide.*

Mean diameter measured in		Difference
lengthwise direction	crosswise direction	
7.64	7.37	0.27
7.12	6.87	0.25
7.31	7.17	0.14
7.53	7.35	0.18
7.41	7.14	0.27

The blood cells are drawn out in a completely uncontrollable way in the length-wise direction of the preparation. This error is eliminated in PRICE JONES' method by measuring the largest and the smallest diameter of the blood cells. The error cannot be eliminated in ocular micrometry.

### Measurement in «wet preparations».

A number of authors measure the diameter in wet preparations with the blood cells suspended in plasma or in other diluting fluids. They are of the opinion that they thereby investigate the blood cells under more natural conditions than if the mea-

TABLE 26.

*MD and s determined in «wet preparations».**Influence of antieoagulants and of time-interval from incision till examination.  
(N = 50, values in mμ).*

Time, in minutes from incision	Blood corpuscles suspended in				
	Serum	Defibrinated plasma	citrate plasma	heparin plasma	oxalate plasma
20					
40	7.95 - 0.66		7.30 - 0.58		
60		7.75 - 0.50	7.40 - 0.65		
80	7.90 - 0.82	8.00 - 0.54			
100		7.60 - 0.54	7.25 - 0.89		
120	7.60 - 0.91	7.75 - 0.59	7.15 - 1.05	7.20 - 0.68	
140		7.45 - 0.67			7.60 - 0.53
160				7.35 - 0.85	
180				7.30 - 0.84	7.85 - 0.61
200					7.57 - 0.73
220				7.30 - 0.89	
240					
260					7.80 - 1.03

surement is done in a blood film. Table 26 gives a series of such measurements, done according to H. C. GRAM's technique. The table shows a certain variation of MD within, as well as between, the series, but, since N in these series is only 50, this may be due to random causes. The standard deviation, however, undoubtedly shows an increase in all series, depending upon the length of time from when the test is taken until the investigation is done. This only expresses in figures what can be directly seen in the microscope, namely that after some time crenation develops, simultaneously with the swelling of other blood cells. These changes are not only due to osmotic disturbances as previously assumed. HEINZE & WOLFF have shown that the two changes may appear simultaneously, and TRØNNBERG is of the opinion that crenation is due to pollution of the blood from the juices of the tissues, fat etc. FÄRHÆUS has shown that the temperature from the microscope lamp may play a role, and JØRGENSEN & WARBURG and LEPEL have shown that the blood cells change during

storage. The measuring of a sufficient number of blood cells will take from  $1\frac{1}{2}$  to 2 hours and during this time the phenomena will appear to a disturbing degree.

### **The difference between different authors normal values.**

In chapter I, fig. 1, it is shown that different authors report different normal values for the mean diameter of the red blood cells. The values fall into groups according to the method employed. In chapter III, page 60 it was shown that whether an oilimmersion objective or a dry lens objective was used caused a difference of 0.4 my in MD. The assistants' different powers of distinction did further account for a difference of 0.4—0.5 my. These two sources of error may account for all the differences observed among the authors in fig. 1 who used the projection method. The three authors in this group who obtained the highest normal values for MD did actually use a dry lens objective, whereas the others used oil-immersion objective. In the two groups where the authors have used direct micrometry, the information regarding the technical details is often incomplete. As far as can be ascertained, however, the variation in these groups as well is covered by the causes of variation mentioned in the previous chapters.

This is not the case regarding the data in the 4th group: measurements done by photography. Experiment no. 6 shows that one may expect that photography will give higher values than micrometry, and the difference which COLLATZ finds when using the two methods may thereby be explained.

But the results which PONDER, and particularly PONDER & MILLAR obtain, fall completely outside what other authors regard as correct values.

These authors measure the size of the blood cells in wet preparations. They use venous blood, with oxalate as anticoagulant. Whether this is of any importance is not known, but in my small series (pg. 79) the samples where oxalate had been added showed the highest values. The authors photograph the cells, using from 18 to 22 exposures. They state that the photographing of each plate takes approximately 2 minutes. The examination of one sample must therefore take 45 minutes or more. During this time, it is probable that the cells will be altered (cfr. pg. 79). They

use monochromatic blue light, and my experiments nos. 9 and 10 show that this definitely gives higher values than when light of other colours is used. They use a dry lens objective, and their degree of enlargement is only 500 x. Finally, they measure the blood cells with a scale graded to 0.10 mm, using a hand lens. I regard it as probable that the personal measuring error (pg. 64) will be larger than usual because their method is more strenuous. It is uncertain whether Brownian movements play any role. PONDER & MILLAR are of the opinion that they do not, whereas COLLATZ abandoned the method because he found that this was a disturbing source of error.

All these conditions will add up, so that PONDER & MILLAR will find higher values than other investigators. These causes should, according to my calculations, bring about an increase in MD of approximately 1.2 my, and might thereby explain the high normal values of these authors.

It has been necessary to discuss these points in detail, since the great variation in the normal values of the individual authors is frequently used as an argument, that measurement of the red blood cells' diameter is too uncertain for the method to have any value.

### Résumé of Chapter V.

The methods of measurement with the ocular micrometer are reviewed. It is shown that these methods give rise to considerable human bias. The methods are so fatiguing that it is difficult to measure a sufficient number of blood cells accurately. In blood films the cells are deformed so that measurements by direct micrometry give incorrect results. In the methods previously mentioned this source of error is eliminated.

Measurement of the red cells in wet preparations is discussed. It is shown that the form and size of the cells change so much during the period of investigation that this method must be discarded.

Finally, the reasons are discussed, why different authors obtain different values for MD in normal subjects. It is shown that the disagreement may be explained by the technical errors of a physical optical nature, and by the causes of human bias, mentioned in chapter IV.

## CHAPTER VI.

### Red blood cell size in pathological conditions.

#### Appearance of pathological frequency curves.

It appears from chapters III—V that it is impossible to obtain an expression for the *true* size of the blood cells with any of the measuring methods mentioned. The best one may expect is to obtain a *relative measure* depending upon a series of technical and personal factors. But when the methods are standardized, and when these factors are given sufficient consideration, the relative size may be determined with satisfactory accuracy.

In experiment no. 11, page 55, MD varies with 0.04—0.10 my around the mean when samples from normal persons are taken over a longer period of time. On page 47 it is shown that the standard deviation does not vary more than allowed by random sampling. Even the concordance with the normal curve measured by  $\chi^2$  analysis is satisfactory (ref. table 33). This does, however, apply to measurements from normal individuals. It remains to be seen whether the technique is satisfactory in pathological cases as well.

PRICE JONES (7) has shown that the frequency curves in pernicious anaemia deviate so much from the corresponding normal curves that one can no longer reckon with a normal distribution of the population. This is also apparent in fig. 13. Series G and H are from patients with chronic hepatitis, the series I and K are from patients with untreated pernicious anaemia. The preparations were, in all cases, taken with an interval of two days during a period when the illness seemed to be stationary.

The curves in each series seem to be related, and they all deviate so much from the normal curve that one can not use this



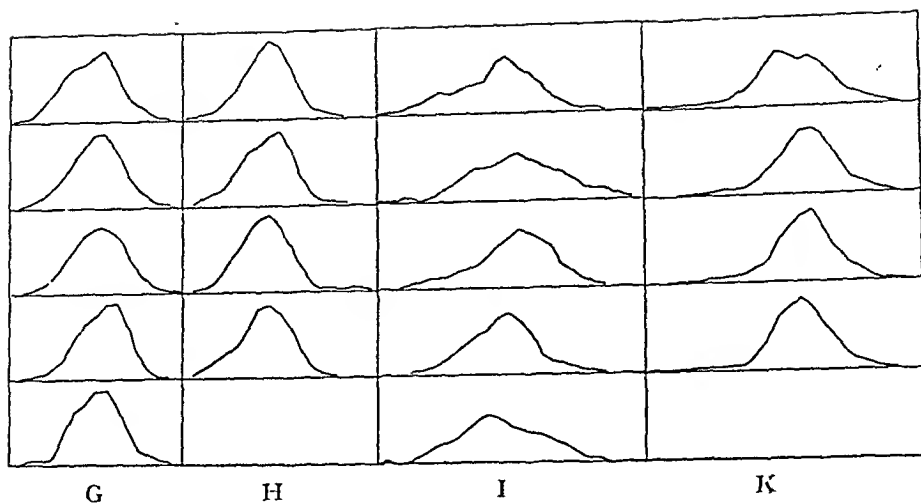


Fig. 13: Frequency curves from pathological blood samples, taken with two days' interval, photographed and measured by the same assistant. G—H Chronic hepatitis. I—K: Untreated pernicious anaemia.

as any measure for their mutual connection. In order to investigate if these curves vary more than allowed for by random causes we may use the following procedure:

*Experiment no. 17:* The frequencies within each variate class in the curves of each series are added together into a grand total which is taken as the exact expression of a hypothetical population. By means of Tippett random sampling numbers, 5 new samples of the same size as the samples which constitute the base for the curves in fig. 13 are drawn from this population. The observed frequency curves, as well as those found by random sampling, are then compared with the curve for the grand total by  $\chi^2$  analysis. The curves which have been found by random sampling are purely theoretical, and technical errors in measurement can not play any role in their case. If the original curves shows equally good concordance with the curve for the grand total as the theoretical curves, there is no reason to believe that the technique of measurement causes a significant variation (table 27).

The number of blood cells measured in this experiment was for each curve 200 in series G, H and I, 500 in series K. Since each blood cell has been measured twice (the largest and smallest diameter), we have in the Tippett series used twice the number

TABLE 27.

 $\chi^2$ -analysis.

Values of  $\chi^2$  for frequency-distributions from fig. 13 and of frequency-distributions found by random sampling, when compared with corresponding grand total frequency-distributions.

Frequency distributions							
G		H		I		K	
Original series	Random series	Original series	Random series	Original series	Random series	Original series	Random series
10.85	14.90	4.31	4.60	11.19	7.04	9.80	3.23
2.67	8.99	4.09	6.94	7.00	11.17	3.10	9.08
5.70	7.64	2.45	2.39	29.02	10.21	8.93	9.80
9.34	9.85	1.74	6.75	14.20	7.76	6.65	11.54
12.44	13.48			16.30	5.05		
41.00	54.86	12.59	20.68	77.71	41.23	28.48	33.65

of frequencies, 400 for the first 3 series, 1000 for the last. The degrees of freedom were 9 in the first two series, and 11 in the last two.

The table shows that the values for  $\chi^2$  for the original frequency curves are no worse than the corresponding Tippett series. Only in one of the series, no. I, is the sum of  $\chi^2$  for the original curves higher than the sum of  $\chi^2$  for the Tippett curves.

When judging this experiment one must be aware that the curves are not independent. There certainly is a correlation between the two diameters measured in the same blood cell. This should give a better agreement with the curve for the grand total, than the frequencies of the Tippett curves.

On the other hand, the original curves have been made with a few days interval, and one can not exclude the possibility that the patient's blood cells may have changed during this period.

But with these reservations the conclusion of the experiment must be that the variance between the observed curves is not greater than what might be expected from random causes, and that the curves therefore presumably represent real conditions in the patient's blood.

## Analysis of heterogeneous frequency curves.

PRICE JONES (7) points out that the frequency curves obtained by measuring blood cells from patients with untreated pernicious anaemia deviate so much from the normal curves that one may assume that the population is not normally distributed. He suggests that they must be constituted by three distinct components. MOGENSEN has worked out a method for analysis of the frequency curves, and has shown that these three components do occur, and MOGENSEN's results have later been substantiated by TÖTTERMANN (2, 3, 4).

The heterogeneity is not so obvious in chronic hepatitis, as clearly shown in fig. 13. But even these curves deviate considerably from the normal curves. It will later be shown that a number of conditions in hepatitis find their best explanation if one assumes that these curves also are heterogeneous. It is therefore reasonable to accept, as a working hypothesis, that the blood cell population in hepatitis is heterogeneous, and to investigate if the observed frequency curves in hepatitis conform with this working hypothesis.

### *Mogensen's method.*

MOGENSEN's starting point is that the diameters of the blood cells in normal persons are distributed normally. When, therefore, the population's mean diameter and standard deviation are known, the population is fully determined by the equation for the normal curve which may be written:

$$f(x-m) = \frac{N}{\sigma \sqrt{2\pi}} \cdot e^{-\frac{(x-m)^2}{2\sigma^2}} \quad (1)$$

where:

$(x-m)$  are the variate classes, characterized by their distance from the mean.

$f(x-m)$  is the number of frequencies in each variate class.  
 $N$  is the total number of frequencies.

$\sigma$  is standard deviation.

$e$  is the base of the natural logarithm.

Equation (1) may be written in logarithmic form:

$$\log f(x-m) = \log \frac{N}{\sigma \sqrt{2\pi}} + \log e \left( -\frac{(x-m)^2}{2\sigma^2} \right)$$

or

$$\log f(x-m) = -\frac{\log e}{2\sigma^2} (x-m)^2 + \log \frac{N}{\sigma \sqrt{2\pi}} \quad (2)$$

But equation (2) is the equation for a straight line of the form:

$$y = -ax + k$$

Equation (2) therefore means that if  $(x-m)^2$  is measured along the abscissae and  $\log f(x-m)$  is measured along the ordinate, then the corresponding values will lie on the straight line with slope

$$-\frac{\log e}{2\sigma^2}$$

If the part of the abscissae which this line cuts off is called  $a$  and the part which the line cuts off the ordinate is called  $b$ , the slope will be  $-\frac{b}{a}$ , and consequently:

$$-\frac{\log e}{2\sigma^2} = -\frac{b}{a} \quad (3)$$

or

$$\sigma = \sqrt{\frac{1}{2} \log e \cdot \frac{a}{b}} \quad (4)$$

Inserting the numerical value for  $\frac{1}{2} \log e$  we obtain:

$$\sigma = \sqrt{0.217 \frac{a}{b}} \quad (5)$$

When  $x = m$ , equation (1) will be

$$f(0) = \frac{N}{\sigma \sqrt{2\pi}} \quad (6)$$

and hence:

$$N = f(0) \cdot \sigma \sqrt{2\pi} \quad (7)$$

$\log f(0) = b$ , and consequently,  $f(0) = \text{antilog } b$ .

Since our class intervals are 0.25 my instead of 1 my, we have to multiply the right hand part of the equation by 4 in order to get the final equation:

$$N = 4 \sqrt{2\pi} \cdot \sigma \cdot \text{antilog } b$$

Inserting the numerical values for  $4 \sqrt{2\pi}$  and  $s$  instead of the unknown  $\sigma$  we obtain:

$$\underline{N = 10.02 \ s. \ \text{antilog } b.} \quad (8)$$

The practical use of the equations may be illustrated with two examples:

In table 28 variate classes and frequencies for a hypothetical curve are given with MD: 7 my,  $\sigma$  : 0.48 my, and frequency sum 400. Since the frequencies have been rounded off to whole

TABLE 28.

*Analysis of frequency curves (Mogensen's method).*

*Normal curve: MD 7.00. s: 0.487. N: 400.*

	Class	n	$(x \div m_0)$	$(x \div m_0)^2$	log n
$m_0:$	5.625	2	1.375	1.90	0.30
	5.875	6	1.125	1.26	0.78
	6.125	16	0.875	0.77	1.20
	6.375	36	0.625	0.39	1.56
	6.625	61	0.375	0.14	1.79
	6.875	79	0.125	0.02	1.90
	7.000				
	7.125	79	0.125	0.02	1.90
	7.375	61	0.375	0.14	1.79
	7.625	36	0.625	0.39	1.56
	7.875	16	0.875	0.77	1.20
	8.125	6	1.125	1.26	0.78
	8.375	2	1.375	1.90	0.30

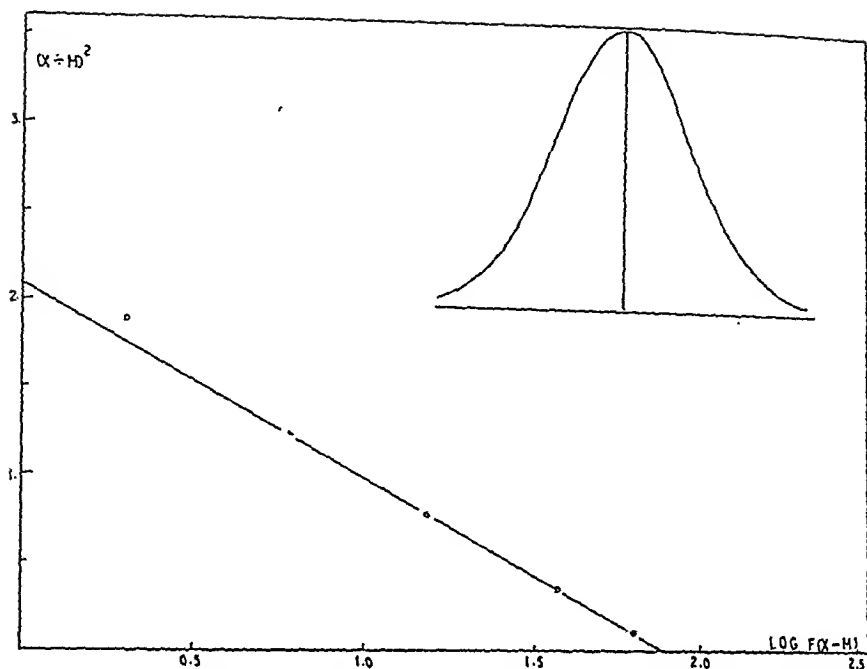


Fig. 14: Analysis of frequency curves. Mogensen's method. Normal curve.

figures the standard deviation as calculated from the frequencies will deviate somewhat from 0.48 and  $s$  will be 0.487. The 3rd, 4th and 5th column in the table show the calculation of  $(x-m)$ ,  $(x-m)^2$  and  $\log f(x-m)$  or  $\log n$ .

In figure 14 the frequency curve has been drawn in the upper right hand corner. The values for  $(x-m)^2$  and the corresponding values for  $\log f(x-m)$  are marked in on the diagram and lie as expected, on a straight line. The value for  $\log f(x-m) = 0.3$  lies outside the line because the frequencies in this class have been rounded off from 1.6 to 2.

From the diagram one reads of  $a = 1.915$  and  $b = 2.11$ .

Inserting these values in the equations (5) and (8) we find:

$s$ : 0.48 my against an actual figure of 0.487 my.

$N$ : 402 against an actual figure of 400.

In table 29 the necessary data are given in the same way for a hypothetical curve composed of two components. One component has mean 7 my, standard deviation 0.48 my and  $N$ : 300. The other component has mean 7.75 my, standard deviation 0.48 my and  $N$ : 100. The frequencies of the secondary component

TABLE 29.

*Analysis of frequency curves (Mogensen's method).*

Composite curve:  $m_0$  7.00  $s_0$  0.477  $N_0$  300  
 $m_1$  7.75  $s_1$  0.496  $N_1$  100

	Class	$(x \div m_0)$	$(x \div m_0)^2$	$n_0$	$n_1$	$n$	$\log n$	$n'_0$	$n'_1$
$m_0$ :	5.625	1.375	1.90	1		1	0.00	1	
	5.875	1.125	1.26	4		4	0.60	4	
	6.125	0.875	0.77	12		12	1.08	12	
	6.375	0.625	0.39	27		27	1.43	28	$\div 1$
	6.625	0.375	0.14	46	2	48	1.68	47	1
	6.875	0.125	0.02	60	4	64	1.81	61	3
	7.000								
	7.125	0.125	0.02	60	9	69	1.84	61	8
	7.375	0.375	0.14	46	15	61	1.79	47	14
	7.625	0.625	0.39	27	20	47	1.67	28	19
	7.875	0.875	0.77	12	20	32	1.51	12	20
	8.125	1.125	1.26	4	15	19	1.28	4	15
	8.375	1.375	1.90	1	9	10	1.00	1	9
	8.625	1.625	2.65		4	4	0.60		4
	8.875	1.875	3.53		2	2	0.30		2

thereby constitute 25 % of the whole population. Even here the standard deviation, when calculated from the frequencies, will deviate somewhat from 0.48 and will be 0.477 and 0.496.

In figure 15, the frequency curve, with the curves of the two components, is drawn in, and as in the previous example the values for  $(x-m)^2$  and  $\log f(x-m)$  are marked in on the diagram.

The values for  $\log f(x-m)$  which correspond to the right hand limb of the curve are marked with • and those which correspond with the left hand limb of the curve are marked with ◦. The values which correspond to the left hand limb of the curve fall on a straight line which gives  $a : 1.90$  and  $b : 1.81$ . The values which correspond to the right hand limb of the curve lie completely outside this line.

Inserting the values for  $a$  and  $b$  in equation (5) and (8) we find the main component's standard deviation: 0.477 my, concordant with the correct value, and the frequency sum: 306 against a correct figure of 300.

The characteristics of the small component are now found by calculating the frequencies in each variate class for a normal curve with  $m : 7.00$  my,  $s : 0.477$  my, and  $N : 306$ . These fre-

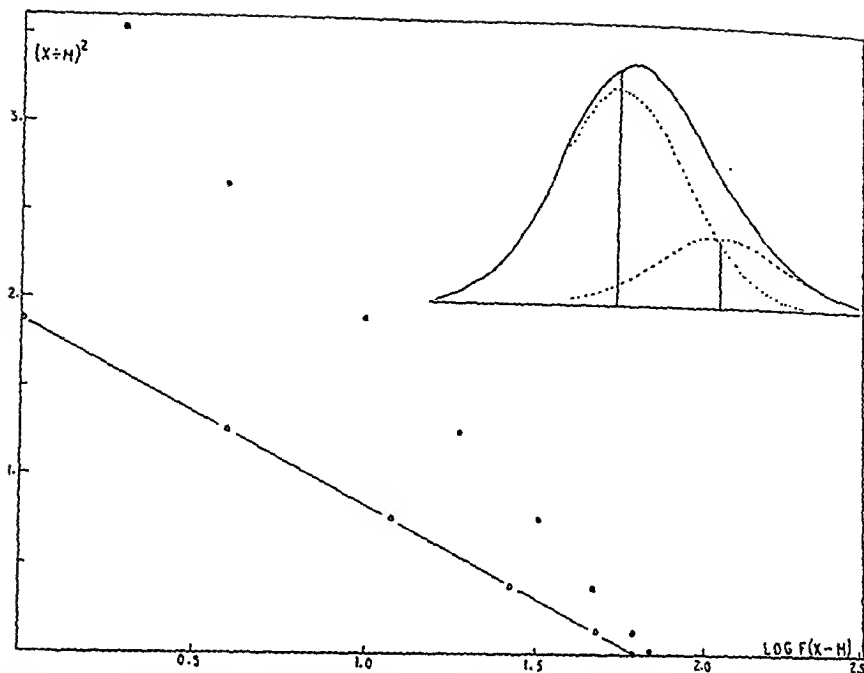


Fig. 15: Analysis of frequency curves. Mogensen's method. Composite curve.

quencies are subtracted from the frequencies in each variate class (table 29 — the two last columns) and the difference is then the frequencies in the small component. The characteristics of the small component are then calculated in the usual way on these frequencies. In our example we obtain the following values for the small component:

m	7.97	my	against	an	actual	figure	of	7.75	my
s	0.46	my	»	»	»	»	»	0.496	my
N	95		»	»	»	»	»	100	

#### *Mogensen's method for the determination of $m_0$ .*

The use of Mogensen's method requires that the mean of the main component, which hereafter is designated by  $m_0$ , is known.  $m_0$  is not identical with the mean diameter MD.

MOGENSEN (1, page 40) indicates the following method for the determination of  $m_0$ :

*«In order first to estimate the mean diameter of the main component, we take advantage of its being symmetrical. This is most easily done by drawing the inversions of the curves on a*



*transparent piece of paper; each inverse curve is now placed on top of the original curve and moved to and fro till it as far as possible fits the original curve. The mean diameter of the main component ( $m_0$ ) is then the mean of two arbitrary abscissae covering one another.»*

My material consist of 342 definitely pathological curves from patients with liver diseases. Mogensen's method for the determination of  $m_0$  was tested on these curves. In 201 cases I was of the opinion that it was possible to determine  $m_0$  by Mogensen's method, while in 141 cases the determination was too uncertain for the method to be used. I then tried to analyse the 201 curves. In 113 cases it was possible to draw a line in the diagrams which allowed a determination of  $a$  and  $b$ . The method failed on this point in 88 cases.

The 113 curves were then further analysed. All of these 113 curves could be broken up into two — and only two — components. The average mean diameter for the one component was  $7.92 \pm 0.44$  my, or very close to the value which I had fixed as a normal value. ( $7.79 \pm 0.24$  my, see table 33). The average mean diameter for the other component was  $8.76 \pm 0.47$  my. In 42 cases the component with the lowest mean diameter was the main component. In 71 cases the component which had the largest mean had the highest number of frequencies. In 93 of the 113 curves, or in 82.4 %, the relative strength of the components was as 70—90 % against 30—10 %.

I found this result very suggestive. Firstly, it was peculiar that the mean of the small component should assemble around the mean diameter, as determined for normal blood cells. Secondly, the difference between the mean diameters in the cases where it was possible to break down the curves was nearly equal in all curves, averaging 0.85 my.

These two facts might possibly indicate that the blood cell populations in patients with liver diseases were composed of two components with difference 0.85 my between their mean diameters, and where one component consisted of normal blood cells. If this was correct, it would be reasonable to assume that the transition between the two groups changed continuously during the course of the illness, and in such extensive material as we

have at hand, one ought to expect an approximately equal number of curves with a distribution of 5, 10, 15 . . . 85, 90 and 95 % of one component. One might then expect to find approximately  $1/3$  of the curves with a relative strength of 70—90 %/30—10 % between the two components. I had found this relative strength in 93 curves or 27.2 % of the whole material. Possibly therefore the missing frequency distributions were hidden among the 229 curves which it had been impossible to break up.

During the analysis of the curves according to Mogensen's method it became obvious that the weakest point of the method lay in the determination of  $m_0$ . If one starts off with an erroneous value for  $m_0$  the values for  $(x-m_0)^2$  will be wrong, and the analysis will fail. In many cases Mogensen's method also gave several possible values for  $m_0$ , out of which only one allowed further analysis.

It was therefore necessary to find another method for the determination of  $m_0$ .

### *The author's method for the determination of $m_0$ .*

By reviewing MOGENSEN's description of the curve analysis and the examples he mentions, it is apparent that the value which he determines by his method with the inverse curve, is the mode, or the highest point of the curve. But fig. 15 shows that the curve is *not* symmetrical around  $m_0$  as mentioned by MOGENSEN. This is only the case when the distance between the mean diameters of the two components is so great that the two components' frequencies do not interfere with each other to any extent.

The preliminary analysis of my material using MOGENSEN's method showed that in those cases where it was possible to break down the curves, the distance between the two components' MD was  $0.85 \pm 0.10$  my. In approximately 78 %, the distance between the means of the two components therefore ought to lie between 0.75 my and 1.00 my, and 93.3 % ought to have less than 1 my between the two means. In fig. 16 a set of curves built up from two normal curves are given with  $\sigma = 0.48$  my and with a distance of 1.00 my between the two mean diameters. The strength of the component with the smallest mean diameter varies from 100 to 0 %. The curve is symmetrical around  $m_0$  when

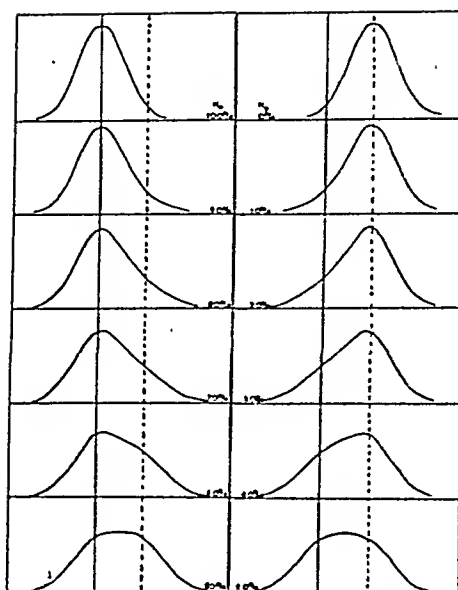


Fig. 16: Form of composite frequency curves, consisting of two normally distributed components ( $\sigma = 0.48$  my) with varying number of frequencies. Distance between the means: 1:00 my.

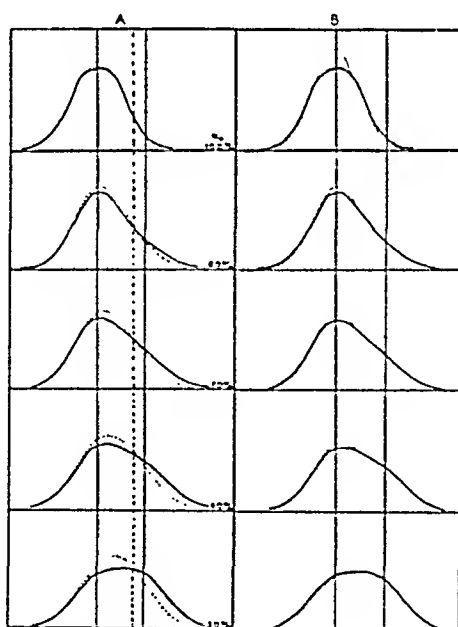


Fig. 17: Form of composite frequency curves, consisting of two normally distributed components. A: Influence of distance between the means. ....: distance 0.75 my. ———: distance 1.00 my. B: Influence of standard deviation: ....:  $\sigma = 0.46$  my. ———:  $\sigma = 0.50$  my.

$N_0$  is 100 %. The curve gradually becomes skew as  $N_0$  decreases and  $N_1$  increases. When  $N_0 = N_1 = 50$  % the curve is again symmetrical, but somewhat platykurtic. Then it becomes skew again, but now as an inverted picture of the previous curves — and finally again symmetrical when  $N_0$  is 0 and  $N_1$  is 100 %. In figure 17 similar curves are given where  $N_0$  decreases from 100 % to 50 %. In the series to the left the distance between  $m_0$  and  $m_1$  is 1.00 my (the fully drawn curve), and 0.75 my (the dotted curve). The series to the right shows how the form of the curve is changed when the standard deviation of the two components changes from 0.46 my (the dotted curve) to 0.50 my (the fully drawn curve).

Fig. 16 and 17 show that the form of the curve is decided mainly by how many frequencies are present in the two components. The distance between the mean diameters of the two components has a certain influence on the form of the curve, the variation in the standard deviation has only a small influence. But it is important to notice that *none of these causes have any considerable influence on the height of the curve or the slope of the left hand limb of the curve.*

This condition is utilized to determine  $m_0$  in the following way:

In fig. 18 curves composed of two components are drawn, each with a standard deviation of 0.48 my. The strength of  $N_0$  varies from 100 % to 50 % with 5 % intervals. The distance between the mean diameters of the two components is 1.00 my (the fully drawn curve) and 0.75 my (the stippled curve). For each curve the MD for the whole curve is marked in with a vertical line.

When  $m_0$  is to be determined, we draw the frequency curve which is to be investigated to the same scale on transparent millimeter paper. The MD of the curve is marked in. We now select the curve on fig. 18 which conforms best with the curve which is being investigated, when the mean diameter of the curve covers the mean diameter of the model curve. It is most important to find a good concordance with the left-hand limb and the height of the curve. If the right hand component of the curve is largest, we utilize the fact that the curves are symmetrical around  $N_0 : 50$  %, and turn the curve so that the right hand limb of the curve may be fitted with the left hand side of the model. When the correct model

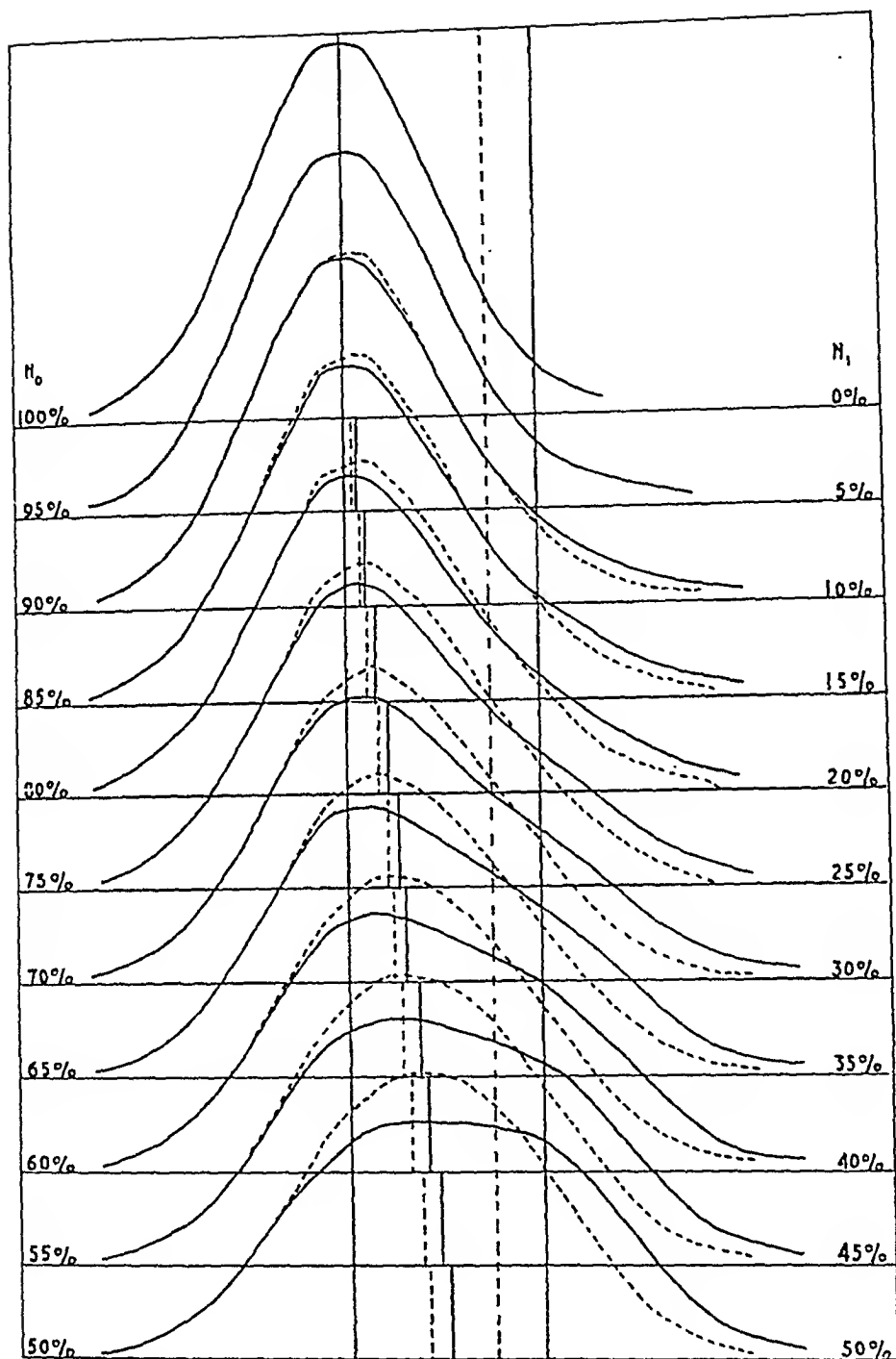


Fig. 18: Model curves used in the analysis of pathological frequency curves. Each curve consists of two normally distributed components, with  $\sigma = 0.48$  my. Distance between the means 0.75 my (the stippled curves) and 1.00 my (fully drawn curves). The number of frequencies in each component is given in the margin. (For practical use see the data given in tables 42—45.)

curve has been found,  $m_0$  is marked in directly on the curve which is being investigated and the analysis is carried out as previously described.

Practical experiments showed that  $m_0$  in this way could be determined satisfactorily in the greater number of cases.

Already when fitting the curve with the model, one obtains quite a good indication of the composition of the curve, but I have found it correct to carry out the full calculation, in all cases. The practical calculations may be facilitated considerably by preparing auxiliary tables.

### *The accuracy of the analysis.*

In order to obtain an impression of the accuracy of the analysis, the following experiment was made.

*Experiment no. 18:* From fig. 18 the curves with  $N_0$  90 %, 80 %, 70 %, 60 % and 50 % were chosen. The frequencies of these curves were taken as the exact expression of corresponding hypothetical populations. By means of Tippet random sampling numbers 4 new samples were extracted from each hypothetical population, altogether 20 series. As in experiment no. 17 the frequency sum was 400 (table 30).

These 20 series, each of 400 frequencies, represent an equal number of samples of the hypothetical populations, and those variations which may occur can only be caused by random sampling.

These 20 series were then dealt with as if they were actually observed curves:

The frequencies were smoothed out after MD and  $s$  had been determined (ref. pg. 66).  $m_0$  was determined in the way described on page 94, and the analysis of the curves was attempted. But before this, they were all mixed so that one did not know the sequence of the curves. The result of the experiment is given in tables 30 and 31. In all cases, the curves were placed in the correct groups. Furthermore,  $m_0$  could not be determined with greater accuracy than  $1/4$  of class interval, or 0.0625 my. On an average  $m_0$  was found to be smaller than the true value and  $m_1$  larger than the true value. The difference  $m_1 - m_0$  therefore became larger than the true value,  $0.86 \pm 0.15$  my against really

TABLE 30.  
Frequency-distributions selected from fig. 18 (in italics) with corresponding random sampling series. Frequencies, MD and s.

	Classes															MD. my	s. my
	5.75	6.00	6.25	6.50	6.75	7.00	7.25	7.50	7.75	8.00	8.25	8.50	8.75	9.00	9.25		
N <sub>0</sub> % 90 %	3	10	23	44	64	74	68	50	32	17	8	4	2	1	0	7.089	0.555
	3	10	17	36	76	82	68	40	37	11	10	8	0	2	0	7.102	0.550
	4	14	24	47	69	71	58	49	35	9	9	8	3	0	0	7.063	0.578
	4	10	19	50	65	73	73	54	36	7	4	3	1	1	0	7.110	0.510
	1	13	27	51	53	88	54	43	35	14	12	5	2	2	0	7.080	0.580
N <sub>0</sub> % 80 %	3	9	21	40	59	69	66	52	38	22	12	6	2	1	0	7.149	0.578
	2	6	27	28	71	74	73	45	37	22	8	5	1	1	0	7.128	0.545
	4	13	18	36	56	71	75	44	49	15	14	3	0	0	0	7.138	0.570
	3	10	29	49	55	72	59	39	35	25	12	9	1	2	0	7.115	0.610
	2	7	18	42	62	82	63	38	40	31	8	6	0	1	0	7.143	0.555
N <sub>0</sub> % 70 %	3	8	19	35	52	62	63	55	42	28	17	8	4	2	1	7.228	0.625
	5	4	18	41	38	57	56	59	51	29	23	9	6	2	2	7.294	0.645
	3	10	23	33	51	67	68	47	37	30	15	11	5	0	0	7.205	0.618
	4	8	12	40	47	59	72	53	40	36	13	12	4	0	0	7.245	0.604
	5	12	12	47	47	58	56	50	53	25	19	7	7	1	1	7.229	0.647
N <sub>0</sub> % 60 %	2	7	16	31	46	57	61	57	48	34	21	11	4	2	1	7.290	0.635
	2	7	13	27	57	59	69	53	53	23	17	12	5	2	1	7.284	0.620
	5	10	16	40	32	57	64	58	47	43	16	7	3	2	0	7.266	0.629
	4	11	24	29	47	51	47	59	50	39	15	13	5	5	1	7.292	0.668
	2	9	18	27	44	55	64	51	47	37	28	11	5	2	0	7.320	0.640
N <sub>0</sub> % 50 %	2	7	13	26	40	52	59	60	53	40	26	13	6	2	1	7.376	0.635
	2	2	10	23	38	53	57	59	66	42	30	7	7	4	0	7.426	0.600
	4	5	16	29	30	56	64	68	48	39	18	15	5	3	0	7.350	0.630
	7	8	17	31	36	56	72	49	44	43	17	15	2	2	1	7.293	0.653
	3	5	17	23	32	53	68	52	53	46	23	13	8	3	1	7.389	0.645

TABLE 31.

*Frequency-distributions selected from fig. 18, with corresponding random sampling series. Parameters and characteristics calculated from data in table 30.*

Parameters:	$m_0$	$m_1$	$s_0$	$s_1$	$m_1 - m_0$	Deviation from	
	7.00	7.75	0.48	0.48	0.75	$N_0$ 0	$N_1$ 0
$N_0 : 90\%$	7.00	8.01	0.43	0.39	1.01	— 10	+ 8
	6.91	7.82	0.48	0.54	0.98	— 20	+ 19
	6.91	7.51	0.46	0.48	0.60	— 42	+ 40
	6.91	7.81	0.48	0.53	0.97	— 25	+ 25
$N_0 : 80\%$	7.00	7.78	0.46	0.49	0.79	0	+ 6
	7.00	7.75	0.46	0.44	0.75	— 20	+ 9
	6.88	7.79	0.48	0.49	0.92	— 20	+ 20
	7.00	8.01	0.47	0.31	1.01	+ 20	— 22
$N_0 : 70\%$	7.00	7.91	0.49	0.46	0.91	— 22	+ 19
	6.91	7.73	0.51	0.51	0.72	— 14	+ 15
	7.00	7.82	0.48	0.44	0.82	— 12	+ 6
	6.91	7.81	0.48	0.43	0.90	— 15	+ 10
$N_0 : 60\%$	7.06	7.87	0.45	0.53	0.80	+ 40	— 45
	6.88	7.73	0.50	0.42	0.86	— 16	+ 20
	6.88	7.88	0.47	0.47	1.00	— 6	+ 8
	7.00	7.90	0.48	0.44	0.90	+ 6	— 11
$N_0 : 50\%$	7.00	7.81	0.44	0.44	0.84	— 5	+ 4
	7.00	7.76	0.51	0.48	0.76	+ 10	— 12
	6.91	7.77	0.49	0.48	0.79	+ 15	— 21
	7.00	7.83	0.50	0.49	0.83	+ 10	— 4
Average:	6.97	7.82	0.48	0.46	0.86	— 6	+ 5
Standarddev.:	0.06	0.13	0.02	0.05	0.15	19	19

0.75 my. This is because in the determination of  $m_0$ , as well as in the later calculation of  $m_1$ , one put too much stress on the peripheral variate classes with few frequencies.

Concerning the determination of  $m$ , as well as  $s$ , the result is poorer in the case of the hi-component than the main component. Firstly, the hi-component usually contains fewer frequencies and the determination is therefore less accurate. Secondly, the frequencies of the hi-component appear after the frequencies of the main component have been calculated, according to the formula for the normal distribution. All random errors will therefore gather in the frequencies of the hi-component.



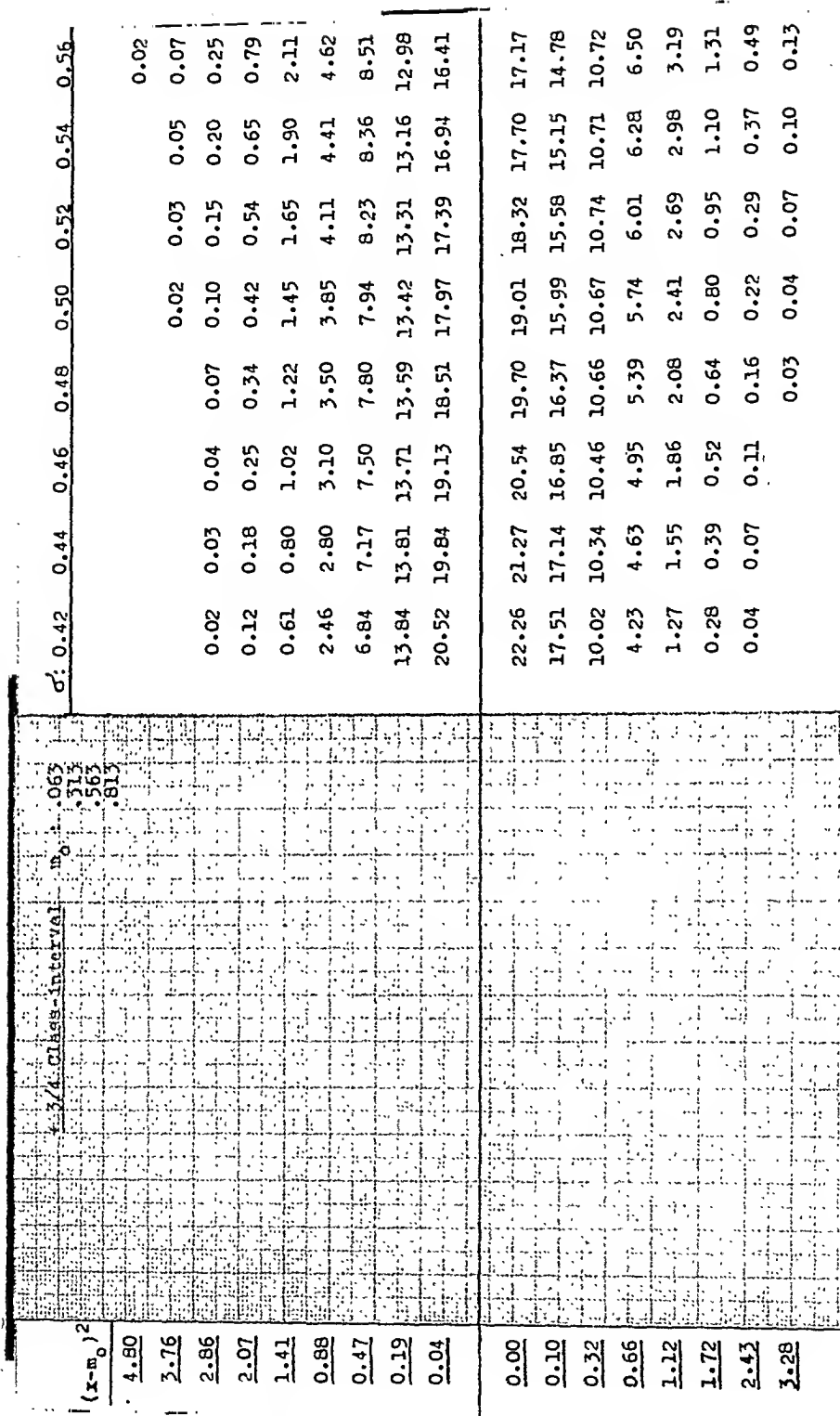


Fig. 19: Scheme used in the analysis of pathological frequency curves.

The degree of variation of the single characteristics is shown in table 31, bottom line. This variation comes in addition to the other errors in the method. The accuracy with which the different characteristics are determined will be discussed later.

*Practical execution of the curve analysis.*

The analysis of the frequency curves demands a considerable calculation work. It may, however, be simplified by using some auxiliary tables. Those functions which frequently reappear are the values for  $(x-m_0)^2$  and the values for the frequencies of the normal curve in the individual variate classes. The scheme shown in fig. 19 was used for the analysis. The values for  $(x-m_0)^2$  are marked in along the left hand border of the sheet. Since it was possible to determine  $m_0$  only with an accuracy of  $1/4$  of the class-interval, we need but 4 schemes where  $m_0$  lies 0,  $1/4$ ,  $1/2$  and  $3/4$  of the class interval from the mean value of the class. On the right half of the scheme the frequencies of the normal curves in each variate class are given in %, for curves with  $\sigma$  from 0.42 to 0.56 my.

The use of the scheme is best illustrated by an example:

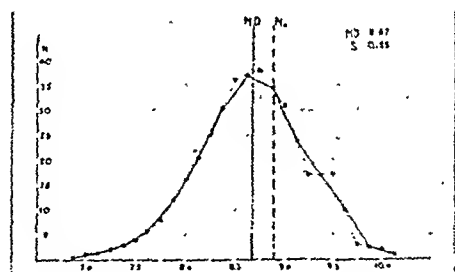


Fig. 20: Analysis of pathological frequency curves. Observed and adjusted curve used in example.

Fig. 20 shows the frequency curve of a case of chronic hepatitis. The observed frequencies are marked with  $\cdot$  and the dotted curve. Based on these frequencies we calculate MD to be 8.67 my, and  $s$  to be 0.55 my. The curve is then smoothed out according to MOGENSEN (pg. 66). The new frequencies are marked with  $\circ$  and the smoothed out curve is indicated by a fully drawn line. The mean diameter is marked in, and the curve is fitted

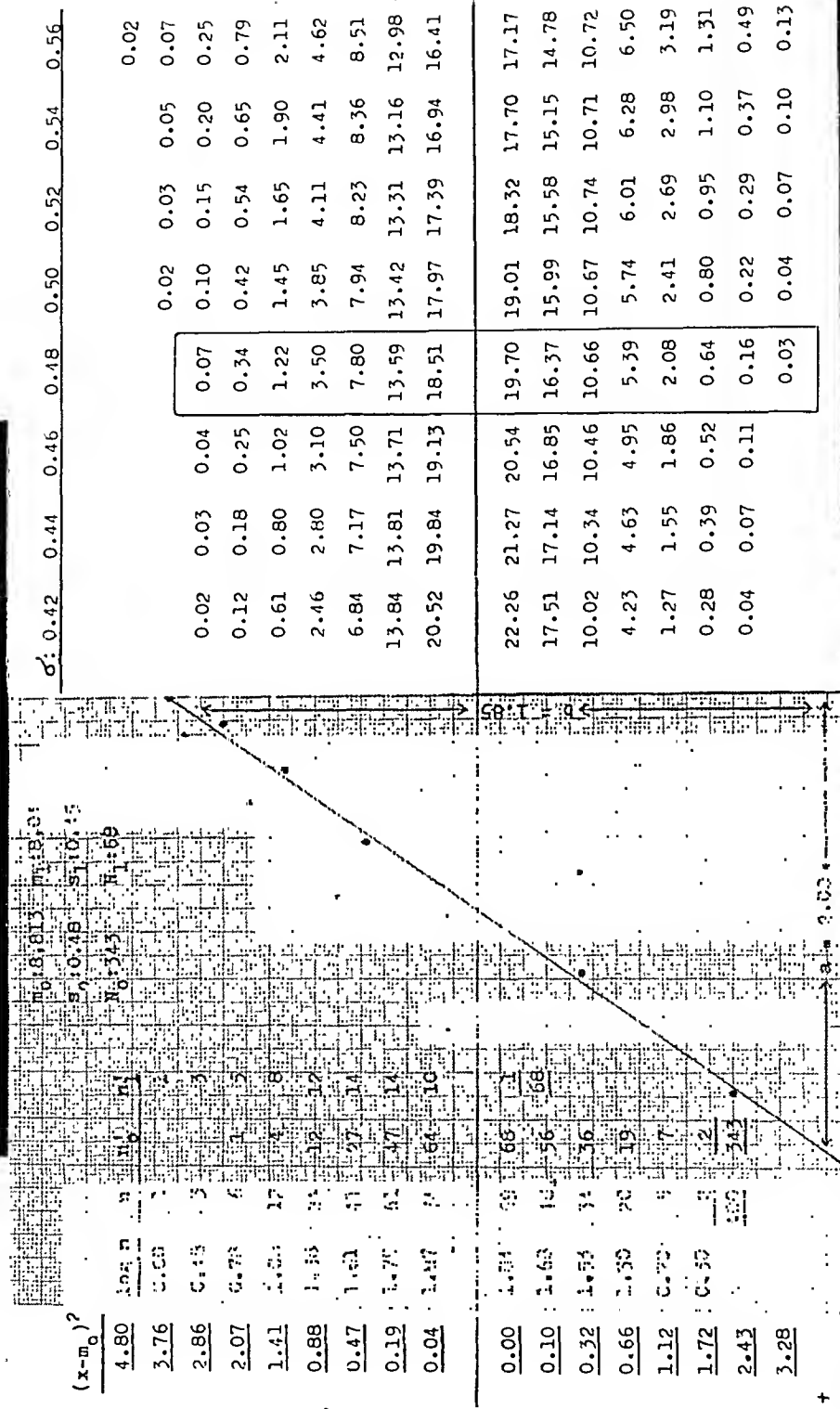


Fig. 21: Analysis of pathological frequency curves. Illustrates the use of the scheme (fig. 19) when analysing the curve in fig. 20.

with the curves in fig. 18.  $m_0$  is found to be 8.813 my. 8.813 my is  $8.625 \text{ my} + 3/4$  of the class interval, and we shall therefore use the scheme in fig. 19. A transparent paper is now placed over the millimeter paper of the scheme and we write in the smoothed frequencies in each variate class (fig. 21, column 3). In a three figure logarithm table we look up the logarithms of the frequencies, and write these in the second column of the scheme. By means of the underlying millimeter paper we mark in the corresponding values for column 1  $[(x-m_0)^2]$  and for column 2  $[\log f(x-m)]$  in the coordinate system. In fig. 21 the values which correspond to the right hand limb of the curve are marked with  $\bullet$  and those which correspond to the left hand limb of the curve are marked with  $+$ . It appears from fig. 21 that all values marked with  $\bullet$  are gathered around a straight line which may be drawn in. By reading off from the underlying millimeter paper we find  $a = 2.00$  and  $b = 1.85$ .

According to equation (5) (page 86) we get

$$s = \sqrt{0.217 \cdot \frac{2.00}{1.85}} = 0.48 \text{ my}$$

According to equation (8) (page 87).

$$N = 10.02 \cdot s \cdot \text{antilog } b$$

Antilog  $b$  is found in the logarithm table to be 71, and consequently:

$$N = 10.02 \cdot 0.48 \cdot 71 = 342$$

The characteristics of the main component are therefore:

$$\begin{aligned} m_0 &: 8.813 \text{ my} \\ s_0 &: 0.48 \text{ my} \\ N_0 &: 342 \end{aligned}$$

In order to determine the bi-component we multiply the frequencies of the normal curve with  $\sigma = 0.48 \text{ my}$  with 3.42. The frequencies are rounded off to the nearest whole figure and entered in the fourth column of the scheme. By subtraction we find the figures in the 5th column, which are the frequencies of the bi-component. The frequency sum of the bi-component should be 57 while we find 68, because we calculate with a normal distribution of the main component instead of the frequencies which really belong

to the sample. From the frequencies of the bi-component we find the characteristics in the usual way to be:

$$m_1 : 8.04 \text{ my}$$

$$s_1 : 0.45 \text{ my}$$

$$N_1 : 68$$

The necessary auxiliary tables are given at the end of the book (tables 42—45).

## Résumé of Chapter VI.

The frequency curves of the blood cell diameters, 2 series from patients with chronic hepatitis and, 2 series from patients with untreated pernicious anaemia, are given in fig. 13. By random sampling it is shown that the variation between the curves in each series is not greater than allowed for by random causes. One concludes that the abnormal curves represent real conditions in the blood of these patients.

It is mentioned that previous authors have shown that these curves are heterogeneous, built up from several distinct components. MOGENSEN's method for the analysis of the pathological frequency curves is reviewed. MOGENSEN's method is tested on 342 frequency curves from patients with liver diseases. In 113 cases the curves could be broken up in two distinct components. The method failed in 229 cases. The cause is a failure to determine the main component's mean diameter. A new method for the determination of this value is proposed. With this new method the analysis succeeds. The accuracy of the analysis is tested by random sampling. The final method for analysis of pathological frequency curves, and the use of prepared auxiliary tables are shown by a practical example.

## CHAPTER VII.

### Final measuring technique. Other haematological methods. Normal values.

#### Measurement of the red blood cell diameter.

The measurements are done in films of capillary blood. The blood films are immediately stained with May-Grünwald-Giemsa stain at Ph. 6.8. All blood films are taken in the morning before breakfast. The films are photographed with the apparatus described on page 45.

#### *The optical equipment.*

*The source of light:* Point-light bulb, 25 w/6 a. The point of image of the light source in the diaphragm opening of the substage condenser.

*Filter:* Yellow green. Centroid of transmission: 5 700—5 350 Å.

*Substage condensor:* N. A.: 1.20.

*Objective:* 1/12 achrom. oil-imm. Leitz. N. A. 1.30.

*Ocular:* Periplanatic Leitz 10 x.

*Degree of enlargement:* 1 000 x.

*Film:* Electrocardiographie paper.

*The measurements* are made with a scale graded to 0,5 mm (i e. 0.5 my). The diameter of the blood cell is the mean of the largest and smallest diameter and is registered in classes with class interval 0.25 my. In order to eliminate the personal error

(page 62 and following) and the assistants' different powers of distinction (page 72) all new assistants were first tested by letting them measure the series A and B in experiment 6. Assistant A's results were chosen as «normal» and no assistant was employed who deviated more than 0.10 my in the determination of MD. Those assistants who had the personal errors mentioned in chapter IV to a marked degree were not employed. As far as possible, one controlled that the same assistant investigated all the preparations from the same patient. In order to avoid suggestion, the preparations were marked with numbers which were not consecutive. The assistant never knew from which patient the preparation was taken.

### *The number of cells measured.*

Previous authors measured a varying number of blood cells, from JORGENSEN & WARBURG who measured 50 blood cells, H. C. GRAM (1), HORNEFFER and others who measured 100, to PRICE JONES (7), MOGENSEN (1), POHLE and others who measured 500, and CROSETTI who measured 1 000. The number of blood cells chosen for measurement frequently appears to be fairly haphazard. PRICE JONES (7) indicates that he measures 500 because «500 cells are a convenient number.» V. BOROS (1) indicates that 200 blood cells are sufficient in normal blood samples, but states, without further substantiation, that it is necessary to measure between 600 and 800 in pathological cases. MOGENSEN (1) chooses the number to be 500 after having discussed the variation of MD in normal persons.

We have here two opposing interests. On the one hand the investigation takes a great amount of time, and an increase from 200 to 500 in the number of blood cells measured necessitates approximately  $1\frac{1}{2}$  hours more work for each preparation. On the other hand, the accuracy of the method increases with the number of blood cells which are measured.

It appears reasonable to me that the problem must be solved by studying the variations in pathological preparations since it is for pathological preparations that the measurement is to be used. It will appear from the previous chapters that it is the form of the frequency curve which is most difficult to bring for-

TABLE 32.

$\chi^2$ -analysis of random sampling series compared with corresponding series selected from experiment no. 17.

Values of  $P$  obtained when measuring different number of cells.

P	Number of blood cells measured						
	50	100	200	300	400	500	1000
1.00							
0.99							
0.98		1		1	1		
0.95		1	1		3	1	
0.90			1	1	1	2	1
0.80	1	2	2	3			2
0.70	2	2	2	2	4	3	1
0.50	5	3	5	4	3	5	1
0.30	3	2	3	3	3		2
0.20	1	2	1	1		2	1
0.10	1	1	1	1	1	2	
0.05	1						
0.02	1	1					
0.01	1						
0.001		1					

ward in a satisfactory manner. In order to answer the question one may use the results from the Tippett-curves in experiment no. 17. Here the Tippett-curves were produced by random sampling from pathological universes and the curves were compared with the universe by  $\chi^2$  analysis. To each value of  $\chi^2$  there is a corresponding value of  $P$  which indicates how well the Tippett-curves conform with the universe. By random sampling from these pathological universes 16 Tippett-curves with 100, 200, 400, 600, 800 and 1000 frequencies, corresponding to 50, 100, 200, 300, 400 and 500 measured blood cells, were selected. The curves



were compared with the hypothetical universes by  $\chi^2$ -analysis. In table 32 the distribution of P in these groups of curves is given. The table shows that the curves which correspond to 50 and 100 measured blood cells have a great spread in the value of P. But already with  $N = 200$  the spread diminishes, and does not improve noticeably when the frequency sum is increased. *I therefore regard 200 blood cells as the most convenient number to measure and this also applies to pathological preparations.* 200 blood cells is the number which gives the greatest degree of accuracy with the least amount of work. The improvement obtained by increasing the number to 500 or 1 000 is not so great that it justifies the considerable amount of extra work entailed.

### *The accuracy of MD.*

In table 33 the normal haematological values are given for 20 normal persons, 10 men, and 10 women. The mean diameter in these 20 preparations is  $7.79 \pm 0.24$  my. In table 12 is shown that assistant A found a difference of 0.71 my in MD by drawing and photography.  $7.79 - 0.71$  gives 7.08 my which agree well with the values found by MOGENSEN (1) (7.15 my) and PRICE JONES (7.2 my), and the difference is therefore assumed to be due to differences in the technique. PRICE JONES, MOGENSEN and HERNBERG have a variation in the diameters of approximately 0.15 my. My variation is somewhat greater, 0.24 my, due to the fact that I measure 200 instead of 500 blood cells, whereby my standard error increases to some extent, and because my measurements have been made by several assistants, each with his individual «factor» (ref. experiment no. 15).

Previous authors decide the accuracy in the determination of the mean diameter by investigating the variation of MD in a series of normal preparations taken simultaneously from the same person. (PRICE JONES (7), MOGENSEN (1) and HERNBERG (1)). It will be more correct to determine the variation in a series of preparations taken over a longer period of time from pathological populations. The variation in MD has two causes. The variation which is caused by random sampling, and the variation which is caused by «technical errors». Experiment no. 17 shows that the variation in pathological samples is not greater than that seen

in hypothetical curves. It may therefore be assumed that the technical error is the same in pathological as well as in normal preparations.

In experiment no. 11 it is shown that in preparations from normal persons, taken over a longer period of time, MD varies with between 0.04 and 0.10 my. This variation is partly due to random sampling, partly due to technical errors. If the total variation is named E, and the technical error z, we can write:

$$E^2 = \frac{\sigma^2}{N} + z^2$$

The best estimation of  $\sigma$  is s, which in normal persons averages 0.45 my. In experiment no. 11 N is 200. From this z may be calculated, and is found to be 0.0948 my, 0.0734 my, 0.0242 my, 0.0948 my and 0.0242 my with an average of 0.0623 my.

In addition to this technical error comes the variation in MD due to random sampling in pathological curves. This is not the same as the standard error in normal curves. An estimate of the random sampling error in pathological curves may be obtained from the Tippet curves in experiment no. 18 (table 30). Calculated from this table the variation in MD around the corresponding parameter is  $\pm 0.0324$  my. The total error in the determination of the mean diameter of pathological samples is therefore:

$$E^2 = 0.0623^2 + 0.0324^2$$

or:

$$E = 0.072 \text{ my.}$$

Approximately 95 % of all observed values for MD should be within  $\pm 2\frac{1}{2}$  E or  $\pm 0.18$  my of the true value and therefore *in the present work we will assume that a difference less than 0,2 my between two observed values of MD in the same patient is without significance.*

#### *The accuracy of s.*

In experiment no. 6 it was shown that s in a series of samples from the same normal person did not vary more than allowed for by random sampling. In order to determine the variation of s in

pathological samples the values in the experiment no. 18 (table 30) may therefore be used. These values for  $s$  vary around their respective parameters with  $\pm 0.023$  my.  $2\frac{1}{2} \times 0.023$  my = 0.06 my which is fixed as the upper limit for the variation in  $s$  between two preparations taken from the same person

It is more important to fix the *upper normal limit* for  $s$ . Table 33 shows that  $s$  in my normal material is  $0.46 \pm 0.02$  my and like PRICE JONES and MOGENSEN i therefore fix the upper normal limit at 0.50 my. Single values from 0.50 my to 0.53 my will be regarded as doubtful, values over 0.53 my as definitely pathological.

### *The accuracy of other characteristics*

Table 31 shows the variation of the other characteristics caused by random sampling and deficiencies in the method used for analysis of the curves. To this variation must be added the variation which is caused by the technical errors. By following the same procedure as before, the variations of the individual parameters are found as follows:

$m_0$  varies with  $\pm 0.087$  my around its parameter

$s_0$  » »  $\pm 0.020$  my » » »

$m_1$  » »  $\pm 0.144$  my » » »

$s_1$  » »  $\pm 0.050$  my » » »

$N_0$  and  $N_1$  varies with  $\pm 5$  % around their parameters.

The variations in  $m_0$  and  $s_0$  are close to the variations previously found in MD and  $s$ . One may therefore fix the same limits for the variations of these two values as were fixed for MD and  $s$ . The variation in the characteristics in the secondary component is so considerable that any direct comparison between values of these characteristics in the same patient has hardly any purpose.

### *The accuracy of the frequency distributions.*

The frequency curves from normal persons may be compared with the corresponding normal curves by  $\chi^2$ -analysis and they then show good agreement (table 33 column P). The pathological curves can not be compared with normal curves. They can, however, be compared with the models in fig. 18 provided the analysis

shows that the population is in agreement with these models. This applies to patients with liver disease, but not to patients with pernicious anaemia. The  $\chi^2$ -analysis in these cases involves rather heavy calculations. I therefore simplified the method in the following way: 6 sets of curves as in fig. 18 were produced where  $\sigma$  varied from 0.42 my to 0.52 my. When a curve had been analysed and its characteristics had been determined, the observed frequency curve was placed over the model with the nearest value for  $N_0$  and  $s_0$ . The difference between the observed and the expected frequencies in each variate class was determined by counting the squares on the millimeter paper. By this method the  $\chi^2$  analysis is considerably facilitated, but we do at the same time introduce assumptions which are not always present:

1) We assume that the two components from which the curve has been built have the same standard deviation.

2) We assume that the distance between  $m_0$  and  $m_1$  is the same as in the model curves, either 0.75 my or 1.00 my. A certain degree of interpolation may however be done.

3) The frequency sum of the main component in the curves found will not always be exactly the same as in the model curve.

Practical experiments show, however, that one obtained good agreement between the  $\chi^2$ -analysis carried out in this way and by analysis carried out by direct computation. By comparing 20 pathological curves where  $\chi^2$  had been calculated in both ways, one found a maximum variation in  $\chi^2 = 1.22$ , and only in 2 of the 20 cases did a difference in  $\chi^2$  result in another value for  $P$ . The  $\chi^2$ -analysis has therefore been carried out in this graphic way, by calculating  $\chi^2$  for 8 variate classes in the case of all curves.

### Other haematological methods.

*Haemoglobin* was determined with a Zeiss-Ikon haemometer adjusted to 100 % = 13.8 gr. In order to calculate mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration, the values found in % for Hb were converted into grams per 100 ccm. blood.

*The number of red blood cells* was counted according to ELLERMAN-ERLANDSEN's method as described by MEULENGRACHT & GRAM.

TABLE 33.  
*Haematological values, 20 normal subjects.*

	MD.	s.	$\chi^2$	P.	Hb. %	Hb. gr.	Red bl.c.	Vol. %	Corp. vol.	MCH	MCC	Corp. Fragility
♀	7.50	0.47	2.14	0.80	92	12.7	4.72	39	32.5	26.9	32.6	0.36/0.44
	7.34	0.43	1.34	0.90	90	12.4	4.64	42	90.6	26.8	29.6	0.28/0.42
	7.71	0.48	2.64	0.70	92	12.7	4.64	37	79.8	27.4	34.4	0.30/0.44
	8.03	0.45	1.63	0.80	95	13.1	4.94	36	72.5	27.1	37.5	0.32/0.46
	7.47	0.44	1.42	0.90	100	13.8	5.05	40	79.4	27.3	34.4	0.32/0.44
	7.91	0.48	1.49	0.90	98	13.5	4.96	39	78.8	27.2	34.6	0.32/0.42
	8.00	0.47	1.92	0.80	95	13.1	5.02	37	74.0	26.2	35.4	0.32/0.46
	7.69	0.43	4.45	0.80	94	13.0	4.32	37	82.1	28.8	35.2	0.30/0.40
	7.69	0.48	4.63	0.80	97	13.4	4.96	39	80.3	27.6	34.4	0.32/0.44
	7.96	0.46	1.78	0.80	101	13.9	5.10	39	76.6	27.3	35.7	0.32/0.46
♂	8.01	0.49	4.75	0.80	105	14.5	5.30	43	81.3	27.4	33.8	0.32/0.46
	8.23	0.45	3.42	0.50	100	13.5	5.40	44	81.6	25.6	31.4	0.32/0.46
	7.95	0.48	1.63	0.80	103	14.3	5.68	44	77.6	25.3	32.5	0.30/0.46
	8.03	0.47	3.44	0.50	94	13.0	5.20	42	80.7	25.0	31.0	0.30/0.46
	7.73	0.46	3.40	0.50	96	13.3	4.66	37	79.5	28.5	36.0	0.30/0.44
	7.75	0.47	3.42	0.50	100	14.0	5.12	39	76.4	27.4	35.9	0.36/0.46
	7.75	0.46	0.21	0.99	105	14.5	5.00	39	78.1	29.0	37.2	0.34/0.44
	7.67	0.45	1.34	0.90	100	13.8	4.96	42	84.6	27.8	32.9	0.34/0.46
	7.41	0.48	1.34	0.90	102	14.1	5.02	41	81.8	28.1	34.4	0.34/0.46
	7.95	0.48	7.20	0.20	106	14.6	5.20	41	78.9	28.1	35.6	0.32/0.46
Mean:	7.79	0.46				13.6	5.00	40	79.9	27.2	34.2	
s ±	0.24	0.02				0.07	0.22	2.5	3.9	1.05	2.06	

*The corpuscular volume (haematocrit value)* was determined with van Allen's haematocrit using the diluting fluid indicated by CHRISTENSEN & WARBURG. A sufficient quantity of diluting fluid was prepared at the beginning of the investigation. It was distributed in bottles of 50 ccm. and sterilized. BETHEL & ROTTSCHAEFER, and BANG & ØRSKOW state that the permeability of the red blood cells in liver disease is changed so that centrifuging with a diluting fluid gives wrong values for the corpuscular volume. Several experiments were therefore performed, comparing the results obtained by van Allen's haematocrit and by a haematocrit where undiluted heparinized capillary blood was centrifuged in capillary tubes. (A similar model of haematocrit has been described by JOSEPHSON (1)). No disagreement between the two methods was found.

FÄRHÆUS (2) and HAHN, ROSS & co-workers have pointed out that the distribution between blood cells and plasma is not the same in venous blood as in capillary blood. Therefore, since all other haematological values were determined in capillary blood, the haematocrit determinations based on venous blood, as for instance with WINTROBE's haematocrit, were not used.

*The fragility of the red blood cells* was determined by mixing blood with NaCl-solutions of known concentration. Macroscopic reading off after 12 hours. The same solutions were used during the whole investigation, and by analysis at the beginning and at the end of the investigation it was shown that the fluids had not changed their concentration.

### *Normal values.*

If we compare the normal values in table 33 with the normal values usually given, the table shows sub-normal values. In Norway the normal values given by LINNEBERG & SCHARTUM-HANSEN are usually accepted as normal. These values conform by the way, well with values given from other countries (WINTROBE, HADEN, VAUGHAN, WHITBY & BRITTON, SCHULTEN and SCHILLING).

LINNEBERG & SCHARTUM-HANSEN give as normal mean values for haemoglobin 15.60 g for men, 14.06 g for women. Red blood

TABLE 34.  
Normal values of different authors.

Author	Haemoglobin in g		Red blood cells in millions		Volume %	
	♂	♀	♂	♀	♂	♀
Linneberg & Schartum Hansen	15.60	14.06	5.27	4.49	45.0	39.0
Lange & Palmer . . . . .	14.35	13.10	4.47	4.01	44.8	41.1
The author . . . . .	14.00	13.16	5.15	4.84	41.2	38.4

cell count: 5.27 mill. for men, 4.49 mill. for women, and vol. % : 45 for men and 39 for women, in all higher values than the corresponding figures in table 33.

Possibly the difference is due to the fact that my investigations were done during the war years 1943—1946. LANGE & PALMER examined 197 students in Oslo in 1943 and found that both the haemoglobin, the number of red blood cells and the volume percentage showed lower values than in previous Nor-

TABLE 35.  
Normal haematological values.

	Mean value	Upper limit	Lower limit
Haemoglobin in g pr. 100 ccm ♂	14.00	15.75	12.25
♀	13.16	14.91	11.41
♀ + ♂	13.60	15.80	11.40
Red blood cell count in mill. pr. cub. mm. ♂	5.15	5.70	4.60
♀	4.84	5.39	4.29
♀ + ♂	5.00	5.70	4.30
Red cell mean diameter . . .	7.80 my	8.35 my	7.20 my
Standard deviation of the dia- meters of the red cells . .	0.46 my	0.50 my	
Volume % (Haematocrit value) .	40	46	34
Mean corpuscular volume . . .	80 cub. my	90 cub. my	70 cub. my
Mean corpuscular haemoglobin in g. $10^{\div 12}$ . . . . .	27.2	30	24
Mean corpuscular haemoglobin concentration (%) . . . . .	34.2	40	29
Red cell fragility . . . . .	0.30/0.46		
Icteric Index (Meulengracht) .		8	

wegian statistics. DAVIDSON, WILCKE, FEIN & REINER have published similar data from Vienna.

In table 34 LINNEBERG & SCHIARTUM-HANSEN's, LANGE & PALMER's and my results are placed together.

Disregarding the values for the number of red blood cells, there is good agreement between LANGE & PALMER's and my values. The cause of the difference in the red blood cell counts is uncertain. The difference may perhaps be due to different apparatuses. All blood counts in this work have however been made with the same apparatus and by the same assistants who determined the values in table 33. I therefore find it correct to use these values as normal values in the present work. My normal values are assembled in table no. 35.

### Résumé of Chapter VII.

The final technique for the measurement of the diameters of the red blood cells is given. Based on random sampling experiments one concludes that measuring 200 blood cells gives satisfactory accuracy with least amount of work, even in pathological cases.

Based on previous experiments the error in the determination of MD is fixed at  $\pm 0.072$  my, that of  $s$  at  $\pm 0.023$  my. Accordingly, a difference less than 0.20 my between two values for MD in the same person is regarded as not significant. The upper normal value of  $s$  is fixed at 0.50 my, single values between 0.50 my and 0.53 my being regarded as doubtful.

The degree of accuracy in the determination of the individual characteristics found by analysis of the curves is discussed. It is shown that the characteristics of the main component are determined with approximately the same degree of accuracy as MD and  $s$ , whereas the characteristics of the bi-component are determined with a considerably lesser degree of accuracy. A graphic method for the  $\chi^2$ -analysis of the curves is described. Finally the author's normal values for other haematological data are given (tables 33 and 35). It is shown that these are lower than those usually indicated. The cause is assumed to be that the present investigation was done during the war.



## **PART II**

### **RED BLOOD CELL DIAMETERS IN LIVER DISEASE**



## CHAPTER VIII.

### The material.

#### Distribution of diseases in individual groups. Occurrence of anaemia and macrocytosis.

##### *The material.*

*The material* consist of 98 patients with jaundice treated in the Norwegian Deacon Hospital, Medical Dept., from June 1943 to August 1946, and of 2 patients with hepatitis treated in the University Clinic, Medical Dept. A in 1947 (patient number 28 and 49).

From this material were excluded those patients whose jaundice was caused by:

Cancer . . . . .	16 patients
Anaemia (pernicious) . . . . .	2 »
Vitium cordis . . . . .	2 »
Febris typhoidea . . . . .	1 »
Icterus from unknown cause or with a too short observation period	5 »

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Total: 26 patients

72 patients then remain.

14 patients had cholecystitis and/or cholelithiasis, without any clinical signs of hepatocellular damage. The diagnosis was made by operation in 10 cases, by X-ray examination in 3 cases, and clinically in 1 case. The remaining 58 patients had hepatitis.

In table 46 the patients are ranked according to the duration of illness. The shortest period was 18 days, the longest more

than  $2\frac{1}{2}$  years. The table shows that it is impossible to draw a border line between acute and chronic hepatitis. There is a gradual transition from one group to another. It is, however, desirable for the further treatment of the material to divide it into one group with the lighter cases and one group with the more chronic cases. I have chosen to place the border line at 90 days. Hepatitis of a shorter duration than 90 days will in the following be designated as acute hepatitis, cases of longer duration will be designated as chronic hepatitis. Purposely no attempt has been made to single out patients who had cirrhosis, even in those cases where this diagnosis was made by operation or autopsy. One of the patients (no. 51) who died, was at autopsy diagnosed as: «sub-acute hepatitis with a transition into yellow atrophy of the liver». This patient is placed in the group: chronic hepatitis. Other cases of yellow atrophy were not found.

It must be stressed that the division into the groups, acute hepatitis and chronic hepatitis, has been made for purely practical reasons. The division does not mean that there is any real difference. I agree with KRARUP & ROHOLM, WANG, BJØRNEBOE & BRØCHNER-MORTENSEN and others that we have the same disease in both groups.

*The aetiology of the disease* is unknown in most cases. 4 patients with acute hepatitis knew about other cases in their nearest circle of acquaintance. In 2 cases (no. 35 and 54) there was a possibility of inoculation hepatitis. 1 patient, no. 27, developed jaundice in connection with a treatment with salazopyrin, so the possibility of intoxication can not be excluded in this case. 4 patients with chronic hepatitis had had previous attacks but with symptom-free intervals in between. 2 patients, no. 14 and 15, had Weil's disease. They are reckoned as acute hepatitis.

*Group A: acute hepatitis*, includes 26 patients. 2 of these had Weil's disease, the others were diagnosed as acute hepatitis. None of these patients had previously had liver diseases, but from 1 to 4 weeks before admission they were taken acutely ill with nausea, vomiting, fever and, a few days later, jaundice. The symptoms disappeared quickly in all these cases, and none of these patients showed any signs of liver damage at the time of discharge from the hospital: Clinically symptom-free, normal values for serum colour

and SR, negative bilirubin and urobilin tests in the urine, negative Takata reaction, normal values for serum albumin and prothrombin.

*Group B: chronic hepatitis*, includes 32 patients. All had been ill for a long period and showed on admission positive signs of hepatocellular damage. 12 patients in this group died in hospital, and 3 shortly after leaving it. Autopsy was carried out in 6 cases.

*Group C: cholecystitis/cholelithiasis*, includes 14 patients. 3 patients had cholecystitis, 3 cholelithiasis, 8 patients had stone in the common bile duct. 10 patients were operated on, 1 died.

During the stay in the hospital, tests were taken at least once a week from all patients for blood count, haematocrit-determination, and for measurement of the diameter of the red blood cells. Some patients were, however, for various reasons, discharged after a short time. In these cases, only one single or a small number of observations are available. Such short series of observations are of little value in a part of the following discussion. The material has therefore been divided into two groups: The patients having from 1 to 3 observations, and those on whom we have carried out more than 3 observations. In the first group we use only the first observation, whereas the others are rejected. The patients in this group are only used in the discussion of some questions. The final classification of the material is as follows:

*Group A: Acute hepatitis:*

Patients with more than 3 observations . . . . .	15	
Patients with 1 observation . . . . .	11	
		— 26

*Group B: Chronic hepatitis:*

Patients with more than 3 observations . . . . .	24	
Patients with 1 observation . . . . .	8	
		— 32

*Group C: Cholecystitis/Cholelithiasis:*

Patients with more than 3 observations . . . . .	6	
Patients with 1 observation . . . . .	8	
		— 14

Total:	72
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At one point in the discussion, I needed blood counts from patients with pernicious anaemia. For this purpose case reports from patients who previously had been treated in the University Hospital, Medical Dept. A, were used.

At another point, I needed frequency curves of blood cell diameters from patients with untreated pernicious anaemia. For this purpose I used blood films from my own collection. These patients have all been treated in hospitals where I have been serving, but they have not been specially examined with the present investigation in mind.

Case-reports, together with the haematologic findings, are collected in table 46.

### Age and sex distribution.

Table 36 shows that whereas the cases of acute hepatitis are distributed fairly evenly between the two sexes, the cases of chronic hepatitis are mostly found in the older age group of women. The same distribution has been found by JERSILD in Denmark.

TABLE 36.  
*Age and sex distribution.*

	Men		Women	
	0—39 years	40—80 years	0—39 years	40—80 years
Acute hepatitis . . . .	5	4	9	8
Chronic hepatitis . . .	1	5	0	26
Cholecystitis . . . . .	1	3	1	9
Chronic hepatitis/died: .	0	4	0	12

### Occurrence of anaemia.

Table 46 shows that 6 of the 26 patients with acute hepatitis, and 23 of the 32 patients with chronic hepatitis have one or several observations where the haemoglobin is lower than 11.4

g/100 cc, the lower border value for normal persons in my material. 2 patients with acute hepatitis and 3 of the patients with chronic hepatitis have subnormal haemoglobin-values during the whole of their stay in hospital.

The number of red blood cells is lower than normal in 4 of the 26 cases with acute hepatitis and in 17 of the 32 cases with chronic hepatitis. The anaemia is usually normochromic and fairly moderate. The lowest value observed in the group acute hepatitis is 9.3 g. Hb. and 3.22 mill. R. b. c., in the group chronic hepatitis 7.6 g. Hb. and 2.5 mill. R. b. c. The mean corpuscular haemoglobin (MCH) is in no case lower than the lowest normal value,  $24 \cdot 10^{-12}$ . 2 patients in the group acute hepatitis and 5 patients in the group chronic hepatitis had one or a couple of observations where MCH is higher than  $30 \cdot 10^{-12}$ , but only one patient had this during the whole course of the disease. Hyperchromic anaemias do not occur regularly in this material.

Increased mean corpuscular volume does not occur in the group acute hepatitis. Values higher than 90 eub. my occur in 4 patients with chronic hepatitis, but only in 1 patient during the whole course of the disease.

### Increase in MD.

Increase in the mean diameter of the red blood cells beyond the upper normal limit occurs in 21 out of 26 patients in the group acute hepatitis, in 21 of the 32 patients in the group chronic hepatitis and in 1 of the 14 patients in the group cholecystitis. The single observation in this group was made in patient number 62, the day after a stone in the common bile duct had been removed and in connection with a collapse.

That the diameter of the blood cells had not increased beyond the upper normal limit in 16 of the patients with hepatitis does not mean that these patients had a normal MD. We will later show that the average increase of MD in hepatitis is approximately 0.7 my. The mean diameter in normal persons may vary between 7.20 and 8.35 my, and it is therefore quite possible that patients may have an increased mean diameter without the value exceeding the upper normal value.

If we calculate the groups' mean value for MD on admission, when the disease is usually at its climax, and for the group acute hepatitis, also when the patients were discharged from hospital, we obtain the following values:

*The mean erythrocyte-diameter in:*

Acute hepatitis on admis. to hosp. (26 patients)	$8.05 \pm 0.26$ my
Acute hepatitis on disch. from hosp. (15 patients)	$7.96 \pm 0.24$ my
Chronic hepatitis on admis. to hosp. (32 patients)	$8.40 \pm 0.45$ my
Cholecystitis on admis. to hosp. (14 patients)	$7.86 \pm 0.25$ my
Normals (table 33) (20 persons)	$7.79 \pm 0.24$ my

*The variance ratio's are:*

Between untreated acute hepatitis and normals . . .	76.05***
Between chronic hepatitis and normals . . . . .	20.22***
Between cholecystitis and normals . . . . .	1.55
Between treated acute hepatitis and normals . . . .	3.95*
Between untreated acute hepatitis and chronic hepatitis	1.14

My material, therefore, shows the same which previously has been shown by a number of authors (see page 15), namely that the mean diameter of the blood cells is increased in hepatitis and is normal in jaundice due to occlusion. The treated acute hepatitis is clinically symptom-free and in concordance herewith, no definite difference between the mean diameters in this group and the normals is found. Finally, there is no significant difference between the mean diameters in the group untreated acute hepatitis and the group chronic hepatitis.

The group cholecystitis has at the time of admission an average mean diameter which does not differ significantly from the normal values. But even in this group we occasionally find macrocytosis during the course of the disease. This occurred in patients nos. 59, 62 and 63 in connection with operation for gall-stones, in patients nos. 64 and 65 in connection with gall-stone attacks with jaundice. The degree of macrocytosis is however slight, and recedes quickly. AAGERUP has shown that the liver parenchyma often shows histological changes in cholecystitis, even in those cases where there are no clinical signs of liver damage.



### Résumé of Chapter VIII.

The clinical material which is used as a basis for the investigations consists of 58 patients with hepatitis and 14 with cholecystitis and/or cholelithiasis.

For practical reasons the patients with hepatitis are divided into one group with lighter cases (duration less than 90 days and resulting in full recovery), and another group with more serious cases. The first group is designated as acute hepatitis, the other group as chronic hepatitis.

The patients have had weekly examinations of the blood during their stay in hospital. The observations (from 1 to 20) of each patient, are collected in table 46.

After a short report on the age-sex-distribution of the material, it is shown that the patients with hepatitis usually have a slight normochromic anaemia, that they have no increase in the corpuscular volume, but that there regularly is an increase in the mean diameter of the red blood cells. In the cases of cholecystitis/cholelithiasis the mean diameter is usually normal or only slightly increased.

## CHAPTER IX.

### The cause of macrocytosis in liver diseases. The theories of «the peripheral cause».

In the introduction it was mentioned that several authors believe the cause of macrocytosis in liver diseases to be found in the peripheral blood. These authors maintain that the changes appear and disappear so rapidly that no other explanation seems likely. They are mostly of the opinion that it is caused by osmotic disturbances, that the blood cells swell and increase in volume by absorbing water.

ENGELSEN reports that he produced macrocytosis in vitro by mixing normal blood cells with icteric plasma.

#### *Engelsen's experiment.*

ENGELSEN mixed his own blood cells with plasma from a patient suffering from jaundice, and observed that his blood cells increased in diameter by 0.34  $\mu$  after 2 hours and 0.44  $\mu$  after 14½ hours.

#### *Experiment no. 19.*

A 20 ccm. syringe was moistened with 2 % heparin solution. Blood from the vena basilica was taken from a patient with chronic hepatitis (icteric index 60) and from a normal person. The blood cells were separated from the plasma by centrifuging for 20 minutes. The plasma was removed by means of a pipette. The blood cells were stirred out in their own plasma and in the other plasma. The number of red blood cells was adjusted to approximately 4.5—4.7 mill. per  $\text{mm}^3$ . and examined, after 2, 24 and 48 hours (table 37).

TABLE 37.

*Blood from normal a person and a patient with icterus. (Both bloodgroup A).  
Blood centrifuged, bloodcorpuscles mixed with own and the other plasma.*

	Time from venipuncture									
	2 hours					24 hours				
	MD	s	Corp. vol.	MT		MD	s	Corp. vol.	MT	
<i>Normal blood:</i>										
Full blood . . . . .	7.51	0.48	86	1.93						
Normal bl. corp. in normal plasma . . . . .	7.82	0.43	83	1.76		7.25	0.54	96	2.08	6.55 0.47 Haemol.
Normal bl. corp. in icteric plasma . . . . .	7.71	0.58	84	1.79		6.71	0.52	96	2.70	6.11 0.49 Haemol.
<i>Icteric blood:</i>										
Full blood . . . . .	8.72	0.67	95	1.58						
Icteric bl. corp. in icteric plasma . . . . .	8.67	0.71	96	1.62		7.18	0.66	112	2.76	6.53 0.57 Haemol.
Icteric bl. corp. in normal plasma . . . . .	8.60	0.63	92	1.58		7.69	0.66	102	2.21	6.81 0.44 Haemol.

The table shows, as in Engelsen's experiment, an increase in MD of the normal blood cells when they are stirred out in icteric plasma. After 2 hours, the increase was 0.20 my. After 24 hours MD decreased to 0.8 my below the normal value.

In concordance with Engelsen's experiment, no significant reduction was observed in the MD of the blood cells from the patient with jaundice when these were stirred out in normal plasma. But the experiment also shows *that the diameter of the normal blood cells increases when they, after the centrifuging, are stirred out in their own plasma.* The increase is 0.31 my, or 0.11 my more than when they are stirred out in icteric plasma. The diameter of the blood cells decreased by storage in all tests. Simultaneously their corpuscular volume increased, showing that the cells gradually attained spherical form. After 48 hours they were so changed that haemolysis occurred when they were centrifuged prior to the determination of the corpuscular volume.

The experiment shows that the increase observed in MD is not due to the icteric plasma as such, but that the increase is an artefact caused by the centrifuging and other manipulations during the experiment. The experiment also shows how careful one has to be when judging experiments in vitro.

#### *Behaviour of blood cells in hypotonic salt solutions.*

*Experiment no. 20:* To get an impression of the way the blood cells are influenced by osmotic disturbances, normal blood cells were stirred out in hypotonic salt solutions, the number of red blood cells in each sample being between 4.16 and 5.00 mill. per mm<sup>3</sup>. The samples were investigated as if they were normal blood samples (table 38).

The table shows that with decreasing concentration of the salt solutions the corpuscular volume of the blood cells increases from 82 cub. my to 120 cub. my whereafter haemolysis occurs. Any simultaneous increase in MD does not occur. The standard deviation increases only slightly, and the concordance between the frequency curves and the normal curves is in every case good, thereby showing that all blood cells are changed to the same extent. This experiment confirms observations made by GUEST & WING: The blood cells behave as if they were surrounded by a semi-



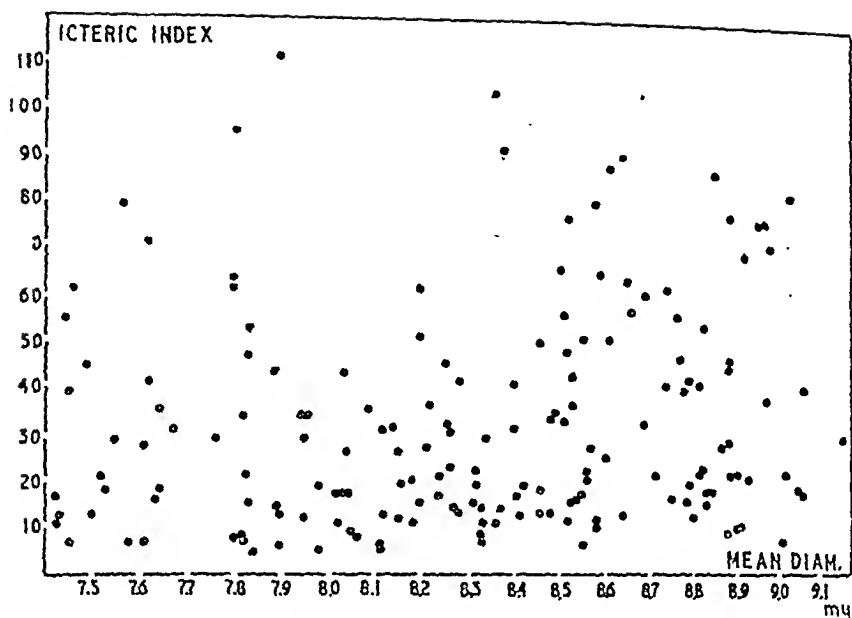


Fig. 22: Corresponding values for icteric index and MD in cases of acute and chronic hepatitis.

permeable *inelastic* membrane. They absorb water until they have attained spherical shape and thereby the largest volume possible for a given surface. When this point is reached haemolysis occurs.

#### *Is the macrocytosis caused by jaundice?*

As mentioned in the introduction, this has been suggested among others by C. GRAM and MEULENGRACHT. A number of other authors have, however, not found any correlation between the icteric index and MD. The corresponding values for the icteric index and MD in my material are given in figure 22. There is no correlation, and jaundice as such, is therefore not the cause of the macrocytosis.

#### *Is the macrocytosis due to osmotic disturbances?*

This is suggested among others by GAMNA, ROSENBERG, BETHEL & RORTSCHAEFER and RODIÑO & BAÑOS.

The changes in osmotic pressure might conceivably be caused by disturbances in the electrolytes and/or by disturbances in the plasma proteins.

*The relations of the electrolytes* have not been specially investigated in my material. But BROCH (1, pg. 66) finds no increase in the size of the blood cells, particularly not in their MD, when the concentration of the electrolytes is reduced. He draws the conclusion that the concentration of the electrolytes changes equally much in the intracellular as in the extracellular fluid. BROCH has further shown that patients with acute hepatitis for the greater part have normal electrolyte values, and on page 122 is shown that these patients have an increase in MD equal to patients with chronic hepatitis.

*Change in the plasma proteins* is more often given as the cause of the macrocytosis. LANGE (2) and PONDER (2) point out that the sedimentation rate, for the greater part, is decided by the content of fibrinogen and globulin in the plasma. One might therefore expect a correlation between SR and MD if changes in the plasma colloids are of any importance. Any such correlation has not been observed in the present material (cfr. table 46).

A simultaneous determination of MD and the content of albumin and globulin in the plasma have been done in 35 cases (table 46). No correlation between MD and total protein could be found, nor any correlation between MD and the globulin concentration in the plasma.

There seems, however, to be a certain correlation between MD and the albumin/globulin-ratio (fig. 23).

Those authors who maintain that there is a connection between MD and the globulin content in plasma are of the opinion that the increase in the size of the blood cells is due to osmotic disturbances. But according to experiment no. 20 one would have to expect a simultaneous increase in the corpuscular volume of the blood cells, and fig. 24 shows no such correlation between the corpuscular volume and the A/G-ratio.

Furthermore, experiment no. 20 shows that the concentration of the salt solutions must be lowered by 0.1 % in order to cause changes which can be measured.

The osmotic concentration of a 0.1 % NaCl solution at 37° is approximately 330 mm Hg. Since the total osmotic concentration of the plasma colloids is only 30 to 40 mm Hg, it is difficult to accept changes in this fraction as the cause of the macrocy-

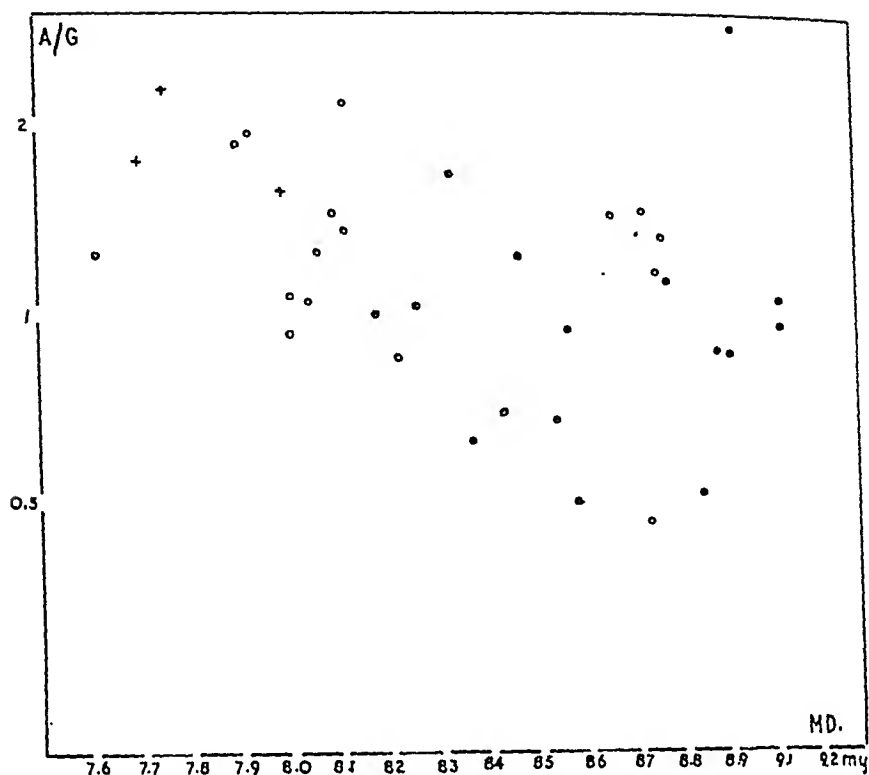


Fig. 23: Corresponding values for MD and Albumin/Globulin ratio.  
 • : Chronic hepatitis. ○ : Acute hepatitis. +: Obstructive jaundice.

toxic. BJØRNEBOE, BRUN & RAASCHOU have shown that if the colloid osmotic pressure in plasma decreases by 11.8 mm. Hg., the patients develop ascites and oedema. Only a few of the patients in this material, and none of the patients with acute hepatitis, had ascites, so it is hardly likely that important disturbances in the colloid osmotic pressure of the plasma were present.

More likely, the correlation which appears between the A/G ratio and MD is due to both changes being an expression for an existing liver damage. The greater this is, the more marked the changes will be. It is worth noticing that the correlation is most marked in the group chronic hepatitis (see fig. 23) where the disturbances in the albumin/globulin-ratio is most pronounced. Fig. 23 shows no definite correlation in acute hepatitis, as far as it is possible to judge from the few observations, and still the increase in MD is as large here as in the group chronic



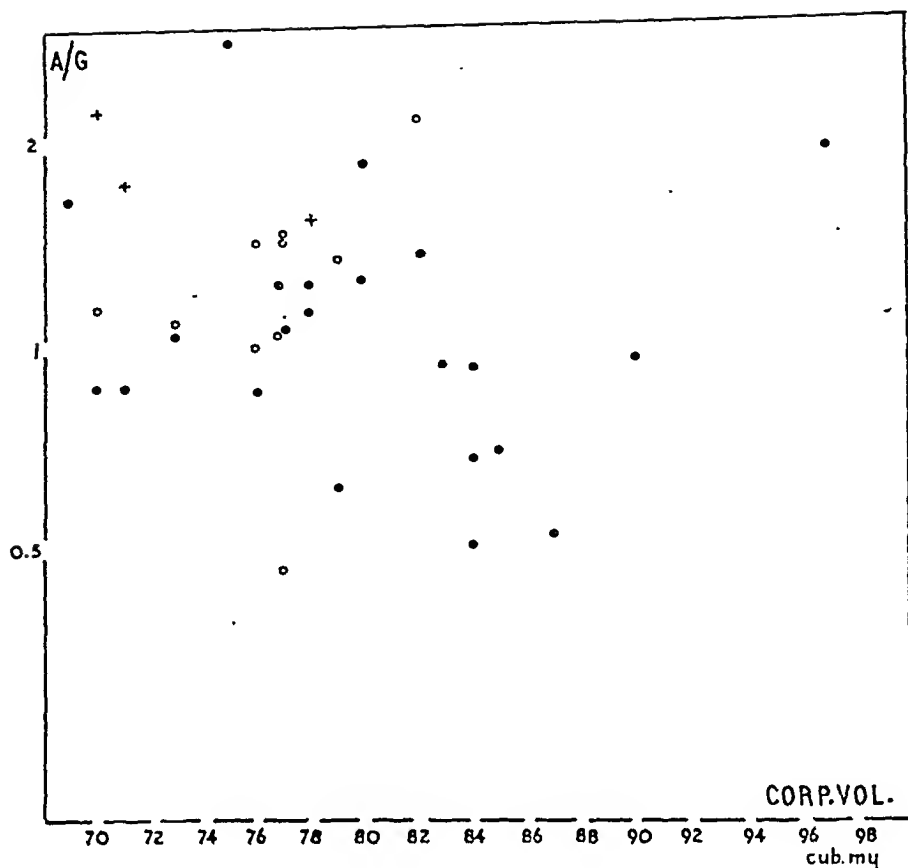


Fig. 24: Corresponding values for mean corpuscular volume and Albumin/Globulin ratio. o : Acute hepatitis. • : Chronic hepatitis. +: Obstructive jaundice.

hepatitis (see literature survey by BODANSKY & BODANSKY page 234).

The conclusion must be that the macrocytosis can not be caused by osmotic disturbances.

Firstly, osmotic disturbances in the plasma of persons suffering from liver diseases do not appear to such an extent that they can explain the observed change in MD. Secondly, experiment 20 shows that the volume should increase without a simultaneous increase in MD, but on page 121 it is stated that exactly the opposite takes place in the present material. The mean diameter of the red blood cells increases *without* a simultaneous increase in the volume. Diagram 25 shows that neither is there any correlation between the mean diameters of the samples and their corpuscular volume, an argument which has previously been pointed

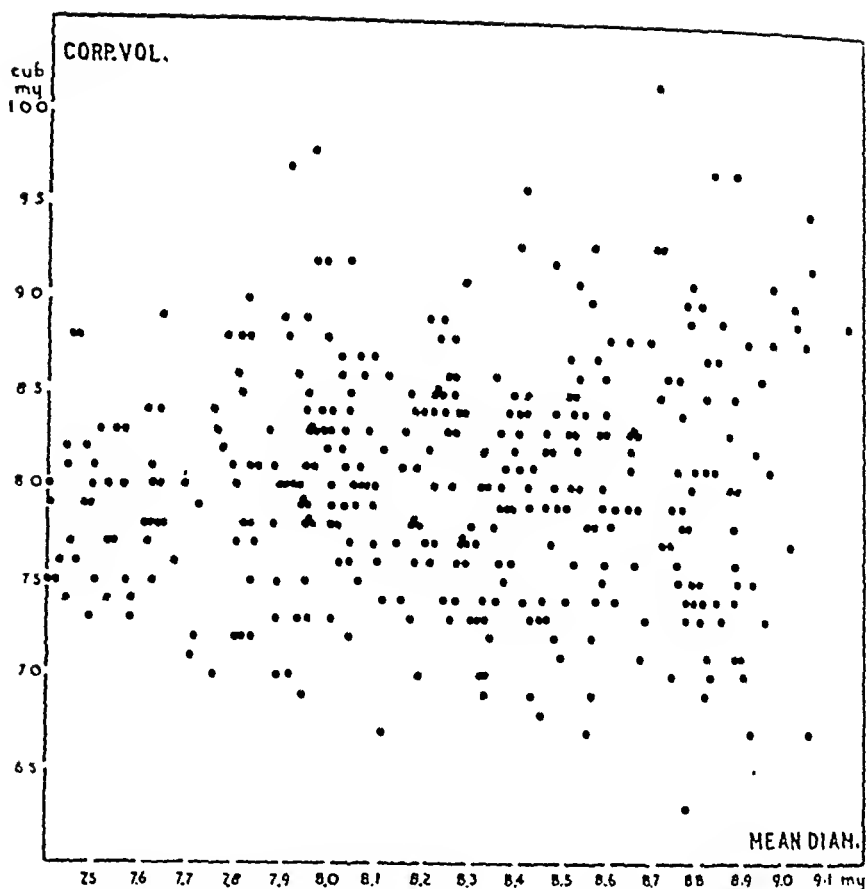


Fig. 25: Corresponding values for Mean Corpuscular Volume and MD.  
(The whole material).

out by HADEN (1) and WINTROBE (3.5). Any dehydration as assumed by JOLLY, is not apparent (normal haematocrit values).

CAPPS is the first to point out a third argument as to why osmotic disturbances can not be the cause: He argues that if the blood cells increase in size because they absorb water, then the haemoglobin concentration in each blood cell would have to decrease. There must be a negative correlation between the MD and MCC. Fig. 26 shows that there is no such negative correlation in my material in those patients who do not suffer from anaemia. Finally, fig. 27 shows, admittedly in a small number of cases, that some samples have increased MD and a simultaneous increase in mean corpuscular haemoglobin (MCH) beyond the upper border value  $30.10^{-12}$ . The regression coefficient of this diagram

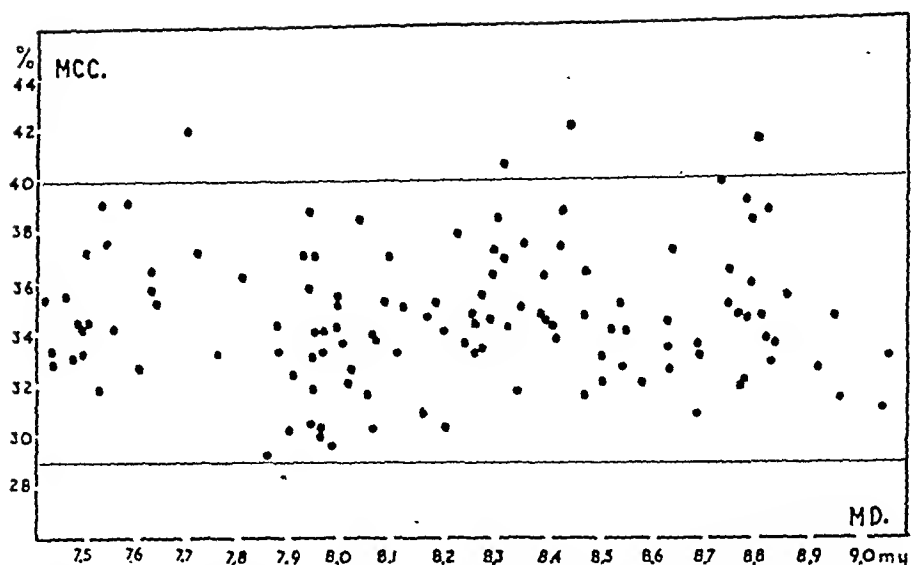


Fig. 26: Corresponding values for MD and Mean Corpuscular Haemoglobin Concentration in patients with hepatitis without anaemia.

is  $+ 0.08$  and thereby shows a very slight tendency to an increase in MCH with increasing MD. Large blood cells, which contain more haemoglobin than normal, can not be a result of osmotic disturbances, so this also disagree with the theory that the causes are disturbances in the peripheral blood.

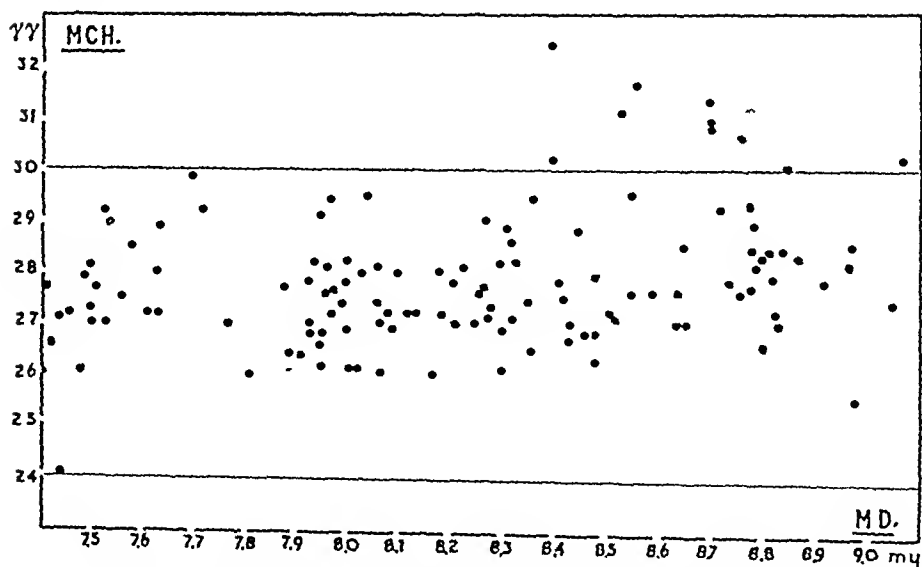


Fig. 27: Corresponding values for MD and Mean Corpuscular Haemoglobin in patients with hepatitis without anaemia.

These arguments definitely conflict with any theory asserting that the already circulating blood cells swell and increase in size in hepatitis. Consequently, the increase in the mean diameter of the blood cells must be due to new blood cells being produced in the bone marrow, and the increase in MD must be caused by a change in the erythropoiesis. But before this view can finally be accepted, one will have to explain the rapid changes in MD observed by previous authors.

### How rapidly does macrocytosis appear and disappear?

As mentioned in the introduction, the main argument for the theory of the peripheral causes is that the changes in MD appear so rapidly that any other explanation is impossible. In order to get an impression of how rapidly the changes take place, we may examine the MD in the 15 patients with acute hepatitis who have been under observation during the whole course of the disease (fig. 28 A). The average decrease in MD is 0.7 my to 1.0 my over a period of 30 to 50 days. The regression coefficient of the diagram is — 0.014 my. This figure conforms well with the corresponding values from MOGENSEN (0.0166 and 0.026) and C. GRAM (0.007 and 0.0103). On page 108 is shown that variations in MD over 0.20 my can not be regarded as being due to technical errors. This border value is surpassed in 14 days.

MOGENSEN mentions that the standard deviation is normal in his cases, and assumes therefore that all blood cells change to the same extent. But then the life-time of the blood cells must be 14 days or approximately  $1/8$  of the life-span usually regarded as correct. And since these patients do not show the usual signs of an increased regeneration as polychromasia, reticulocytosis or nucleated red blood cells in the peripheral blood, there is no reason to assume that the life-time is shortened in such a way.

The point at issue is that these patients have a normal standard deviation. But out of 6 of Mogensen's patients with hepatitis one patient only had a value for  $s$  lower than 0.50 my, and one of his patients had  $s : 0.53$  my which is his border value for normal values.

Fig. 28 B shows the standard deviations for my patients with acute hepatitis. The diagram shows that the standard deviation

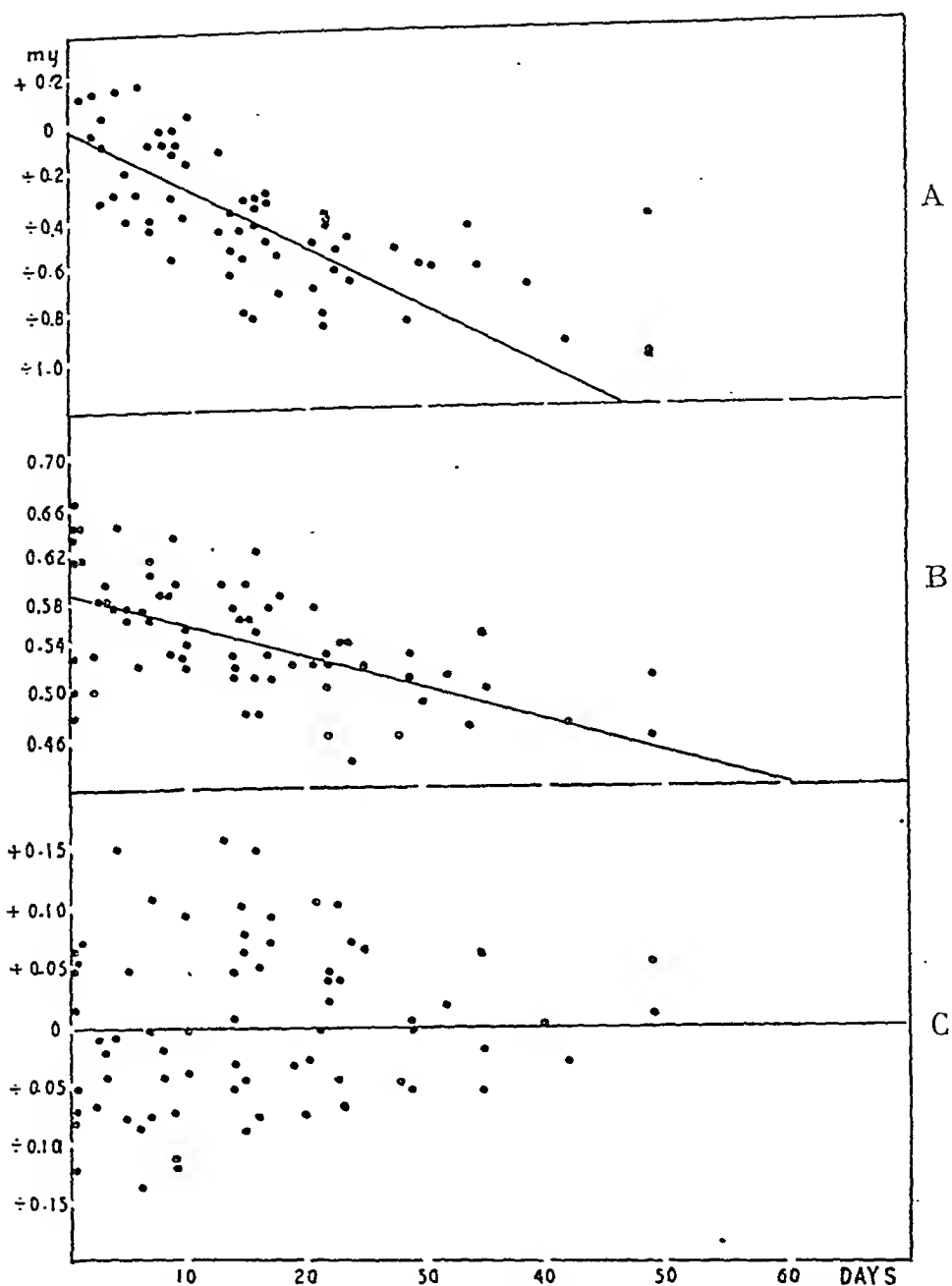


Fig. 28: The size of the red blood cells during the healing of acute hepatitis. A: The gradual reduction in MD. B: The gradual reduction in  $s$ . C: The gradual reduction in the skewness of the frequency curves, measured by the difference between the mode and the mean.

is definitely too high when MD is at its highest, the greater number of the values being higher than 0.53 my. When the disease recedes and MD becomes normal, then  $s$  also decreases, but not with the same rapidity as MD.

In fig. 28 C the corresponding values for the skewness of the curves, measured by the difference between the mean diameter and the mode are gathered. It is obvious that the curves are skew when MD is increased, and that they become symmetrical, or nearly symmetrical when MD becomes normal.

Diagram 29 gives all corresponding values of MD and  $s$  in the whole material. Both in the groups acute hepatitis and chronic hepatitis a large number of curves is found with normal values for MD, below 8.35 my. But only a small number of curves have a normal  $s$ , lower than 0.50 my. Even if we choose the border for a normal  $s$  to be 0.53 my, the standard deviation of the greater number of the curves is pathological.

These simultaneous increases in the standard deviation and the mean diameter cancels the argument of MOGENSEN. When the standard deviation increases and the curve becomes skew, then the increase in MD may be due to a group of new large blood cells appearing and mixing with those already circulating in the blood. The increase in MD is then no longer dependent only upon the number, but also on the size of these new blood cells. If the difference in size is sufficiently great, then a comparatively small number of new blood cells will draw the mean of the whole population upwards, and may thus explain the increase in MD. That this is what really occurs in liver disease has already been suggested by C. GRAM in 1883, and again by HAMMARSTEN & STÄHLE in 1943. A similar cause for macrocytosis has been found in other conditions by ROSENQUIST and SJÖWALL.

*The present data may therefore indicate that the increase in the mean diameter is due to large new blood cells entering the peripheral blood, and it is natural to assume that these new blood cells originate in the bone marrow.*

Another possibility, suggested by BARCROFT, KRACKE, and LANGE, that the large blood cells originate from the spleen or other peripheral blood depots need not be considered. In the case of chronic hepatitis the macrocytosis may last for weeks and months, and one must assume that such depots would be emptied

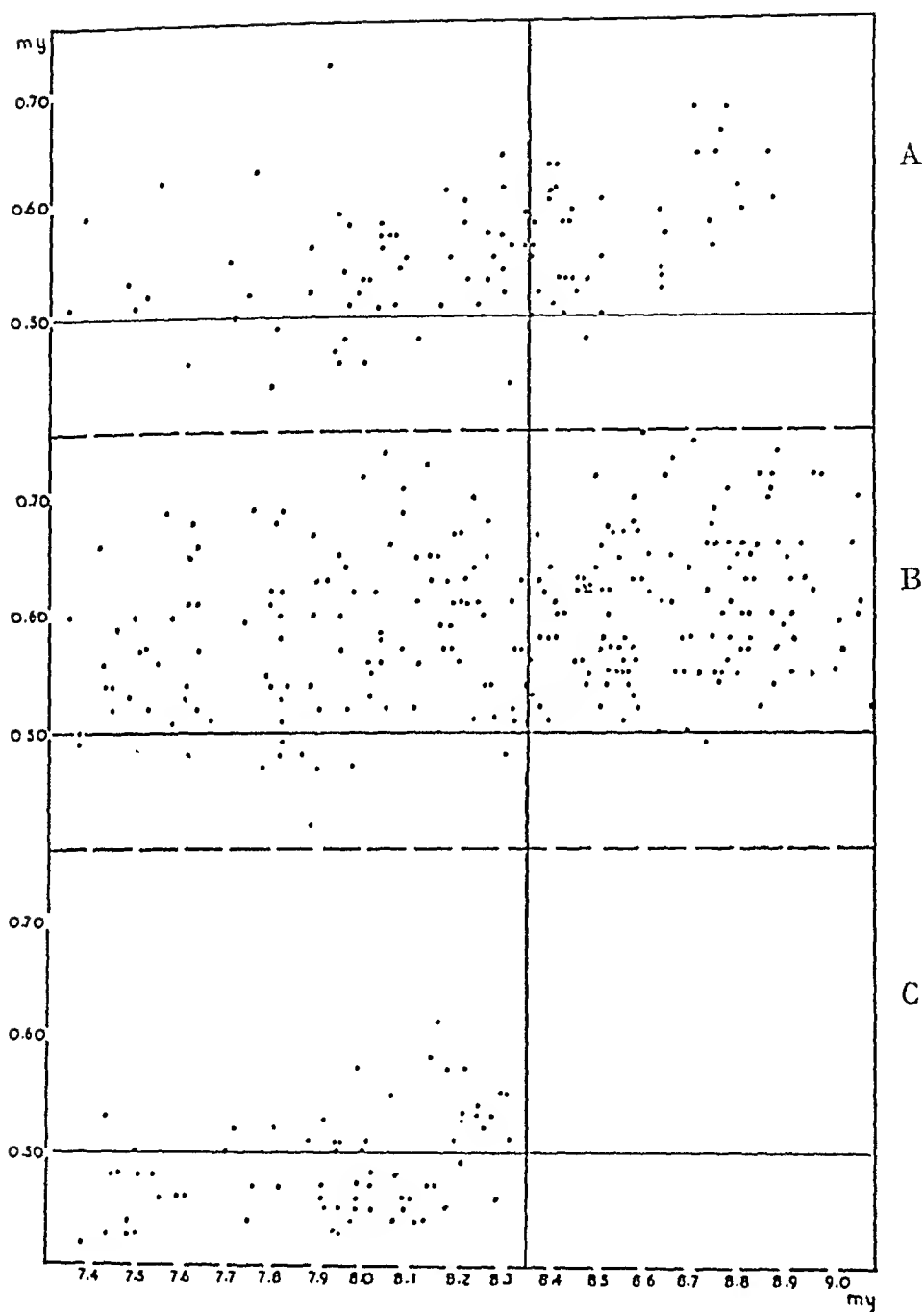


Fig. 29: Corresponding values for MD and s. A: Acute hepatitis. B: Chronic hepatitis. C: Obstructive jaundice.

rapidly. Neither can one assume that the increase is caused by reticulocytes of large size which do not mature and decrease in size in the peripheral blood (PLUM). The highest value observed for reticulocytes in my material is 1.4 %, and it will be shown that their number is insufficient to explain the increase in MD.

### Résumé of Chapter IX.

In experiment no. 19, blood cells from a normal person were mixed with icteric plasma. It is shown that this results in an increase in the diameter of the blood cells, but that this increase is an artefact.

In experiment no. 20, normal blood cells were mixed with hypotonic salt-solutions. The corpuscular volume of the blood cells increased without a simultaneous increase in MD.

The possibility that the increase in MD might be caused by osmotic changes in the peripheral blood is discussed. It is shown that this explanation of the phenomenon does not agree with the present facts.

It is shown that an increase in MD is followed by a simultaneous increase in the standard deviation of the frequency curves, and that the frequency curves become skew (fig. 28). This, together with the lack in signs of any increased regeneration, makes it probable that the increase in MD is due to new, and considerably larger blood cells than normal originating from the bone marrow, and that this is the explanation of the increase in the mean diameter of the blood cells observed in liver diseases.



## CHAPTER X.

**The cause of macrocytosis in liver diseases.**

**The theories of «the central disturbance».**

**The population is heterogeneous and consists of two components.**

In the previous chapter it was shown that the macrocytosis in liver diseases hardly is caused by disturbances in the peripheral blood. It was shown that the rapid variation in MD may best be explained by presuming that the blood cell population is heterogeneous and consists of normal blood cells and a new type of large blood cells, added from the bone marrow.

If this assumption is correct, then the blood cell population must consist of at least two components, one of which is normal. If we further assume, as a working hypothesis, that the other component consist of cells, also of a fixed size, then the MD of the total population will be determined by the number of pathological cells which at any time are present in the blood. The highest value of MD will be obtained when there are only pathological cells in the peripheral blood.

MOGENSEN has shown in a convincing way that there is such a heterogeneity in pernicious anaemia, and TOTTERMANN finds a similar heterogeneous population in bothriocephalus anaemia. The cells of the pathological component are, in both cases, distributed normally around their mean, with a standard deviation of approximately normal size. If we assume that similar conditions are present in liver disease, then a population which only consists of pathological large cells has a normal standard deviation. In the present material we have 25 samples with increased MD and

simultaneously normal standard deviation (MD over 8.35 my, s lower than 0.53 my). The mean diameter of these 25 samples is  $8.56 \pm 0.19$  my. If we assume that these 25 samples only consist of macrocytes, then the diameter of the macrocytes should be approximately 8.56 my or about 0.75 my larger than the normal blood cells.

On page 85 the analysis of the frequency curves in this material according to Mogensen's method is described. It was possible by means of his method to split 113 curves into two components, and the distance between the diameters of the two components was on an average 0.85 my. It may be worth while to stress that we here, in two different ways, arrive at nearly the same value for the mean diameter of the possible pathological component.

The standard deviation of a heterogeneous curve is determined by the standard deviation of the individual components, by the number of frequencies in each component, and by the distance between the mean diameters of the two components. The greatest standard deviation in a heterogeneous curve is observed when the two components are of equal size (see fig. 16). A heterogeneous curve consisting of two normal curves with  $s = 0.50$  my, with equal number of frequencies, and where the distance between the mean diameters of the two components is 0.75 my will have a standard deviation = 0.68 my. This is therefore the upper border value for s if the reasoning is correct. Fig. 29 shows that this border value was exceeded only by 20 out of 342 pathological curves or in 5.8 %. The highest value observed for s is 0.80 my. This is in contrast to my curves for untreated pernicious anaemia where s goes up to 1.16 my and only in one case is lower than 0.80 my (see table 40).

It may therefore be justifiable to advance the following

#### *Working hypothesis:*

*The macrocytosis in liver diseases is caused by the blood cell population being heterogeneous. The population consists of two distinct components, each with its characteristic mean diameter. The distribution around the mean of each component is normal*

and has a normal standard deviation. One component consists of normal blood cells.

This working hypothesis may be tested by trying to analyse the observed frequency curves as described in Chapter VI.

### *The final analysis of the frequency curves.*

The present material contains altogether 412 frequency curves from patients with liver diseases.

70 of these curves had a normal MD, a normal  $s$ , and satisfactory concordance with the normal curve by  $\chi^2$  analysis. These 70 curves were therefore regarded as normal. In the case of the other curves an analysis was attempted.

When  $m_0$  is determined by my method, the curves are compared with the models in fig. 18. When the asymmetry of the curve is slight, there may be a doubt as to which of the two components is the main component. In these cases both possibilities were tried. We then obtained two solutions. By comparison with the other curves from the patient, it was usually obvious which solution was the correct one. In cases of doubt, a new blood film taken just before or just after the doubtful sample was stained, and the question was answered by following the patient more closely in the doubtful period. None of the curves were rejected, but those curves which have two solutions are in table 46 marked with a line under the figure for MD. This concerns altogether 39 curves, or 9.5 % of the total material.

In 8 cases it was impossible to decompose the curves, in spite of their being definitely pathological. The probable composition of the curves was determined by comparing them with fig. 18. These samples are in table 46 marked with «graphie».

The result of the analysis may then be summed up thus:

Successful analysis with 1 solution . . . . .	295 curves	71.5 %
Successful analysis with 2 solutions . . . . .	39 »	9.5 %
Normal curves, not heterogeneous . . . . .	70 »	17.0 %
<hr/>		
Successful analysis . . . . .	404 curves	98.0 %
Unsuccessful analysis . . . . .	8 »	2.0 %
<hr/>		
	412 curves	100 %

The result of the analysis is given in table 46. We have here introduced the following terms:

The characteristics of the component which lies to the left in the curve has the affix  $_l$  (left), thus:  $m_l-s_l-N_l$ .

The characteristics of the component which lies to the right, and which consist of large blood cells, have the affix  $_r$  (right), thus:  $m_r-s_r-N_r$ .

Where the largest component, irrespective of cell-size, is concerned the affix  $_0$  is used, thus:  $m_0-s_0-N_0$ .

The characteristics referring to all frequencies have no affix.

The satisfactory result of the analysis shown in table 46 is not in itself any proof that the blood cell population really is built up by two distinct groups. The whole analysis is based on the determination of  $m_0$  — and the method for the determination of  $m_0$  is based upon the assumption that the population consists of two components. The proofs, that the analysis reflects true conditions, must be searched for in circumstances independent of the working hypothesis.

### Conditions confirming the heterogeneity of the population.

*A: One component consists of normal blood cells.*

This is a condition in the working hypothesis. But then the observed mean diameters for the cells of this component must not deviate significantly from the mean diameters in normal persons. Table 46 shows that

the average value for  $m_l$  is:

Group: Hepatitis acuta . . . . .	7.93 $\pm$ 0.21 my (15 pas.)
Hepatitis chronica . . . . .	7.91 $\pm$ 0.37 my (24 pas.)
Cholecystitis . . . . .	7.86 $\pm$ 0.25 my (6 pas.)
Group: Normals (table 33) . . . . .	7.79 $\pm$ 0.25 my

Variance ratio  $\frac{\text{between groups}}{\text{within groups}}$  is 1.51, which is *not significant*.

*B: The pathological component has a fixed mean diameter.*

It is assumed in the hypothesis that the macrocytosis is due to a new class of large blood cells which originate in the bone marrow. This class of new blood cells should therefore have a definite mean diameter.

The average value for  $m_r$  is:

Group: Hepatitis acuta . . . . .	$8.65 \pm 0.20$ my (15 pas.)
Hepatitis chronica . . . . .	$8.58 \pm 0.50$ my (24 pas.)
Cholecystitis . . . . .	$8.63 \pm 0.12$ my (6 pas.)

The variance ratio  $\frac{\text{between groups}}{\text{within groups}}$  is 6.3\*, somewhat poorer than in the preceding case, but not even here significant.

*C: The curves show a satisfactory concordance with the theoretical curves.*

We can by  $\chi^2$  analysis, determine whether the form of the curves is in concordance with the theoretical curves. All the found frequency curves which could be decomposed into 2 components were therefore compared with the corresponding theoretical curves in fig. 18. The  $\chi^2$  analysis was carried out by means of the graphic method described on page 109, Using 8 variate classes in all curves. The working hypothesis and the technique of the analysis introduces 5 constraints in the method:

- 1) that  $MD - m_0$  is the same as in the model curves in fig. 18.
- 2) That  $N_0$  is the same as in the corresponding model curve.
- 3) that the frequencies in the main component are normally distributed around  $m_0$ .
- 4) that those frequencies which do not belong to the main component are gathered on one side of  $m_0$ .
- 5) that  $N_1 + N_r$  is 100 %.

We have therefore  $8-5=3$  degrees of freedom. The found values for  $\chi^2$ , altogether 334, ought be distributed in concor-

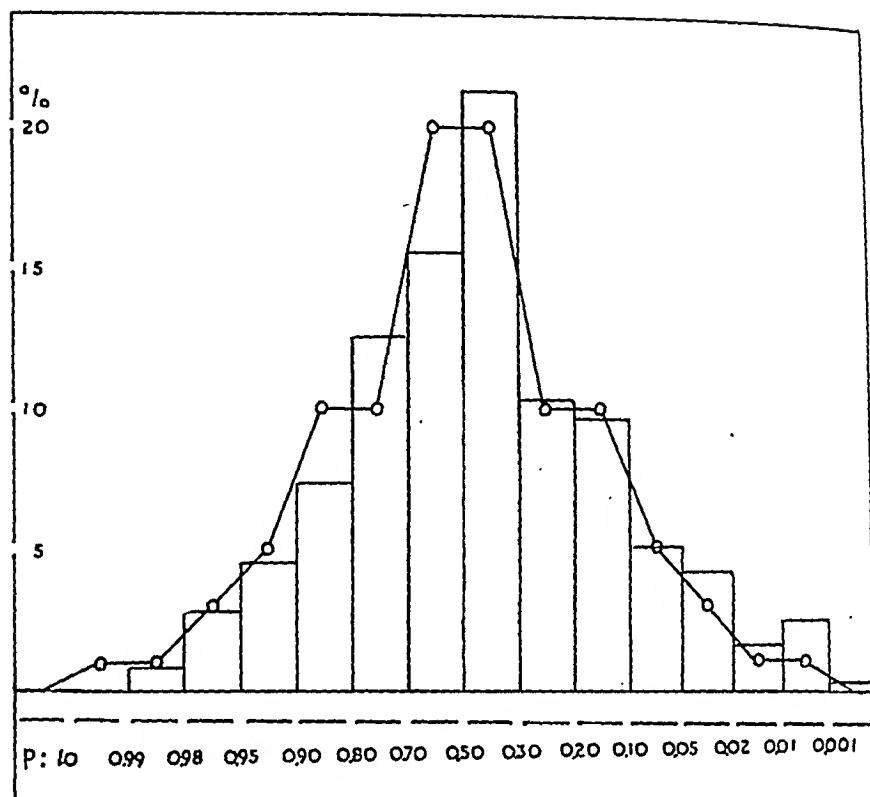


Fig. 30:  $\chi^2$ -analysis. The concordance between observed frequency curves and the theoretical populations. Histogram: Observed values of P. Curve: Expected distribution of P. Number of curves examined: 334.

dance with the theoretical distribution of  $\chi^2$  for 3 degrees of freedom. Fig. 30 shows that there is a very good concordance between the found and expected values.

*D: The number of pathological blood cells varies continuously.*

Table 46 shows that such a regular variation really does occur. By way of illustration the observed curves for 3 patients with acute hepatitis and one patient with chronic hepatitis are given in fig. 31. In each curve the normal curve of the main component and the frequency curve of the bi-component as it appears when the frequencies of the main component are subtracted from the frequency sum is drawn in.

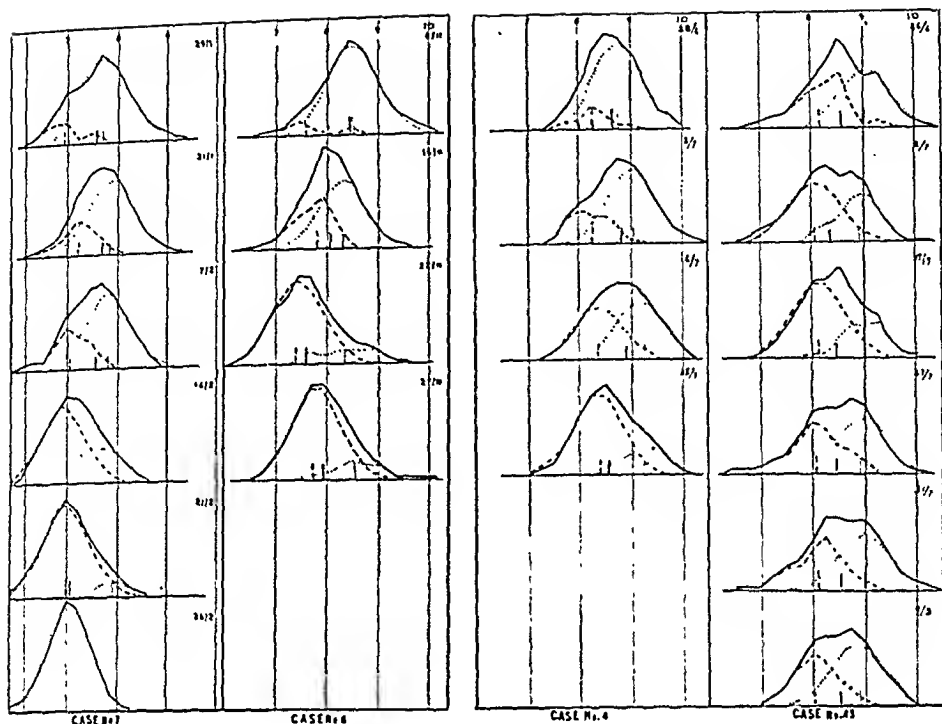


Fig. 31: Observed frequency curves, with the relative strength of the two components, during the course of acute hepatitis (case no. 7, 6, and 4) and chronic hepatitis (case no. 45). Data from table 46.

Fig. 31 shows that in acute hepatitis the right hand component decreases whereas the left hand component increases and finally becomes dominant. In chronic hepatitis the relative strength of the two components varies around the 50 % level during the whole observation-time. Fig. 31 further shows no difference in the form of the curves in untreated acute hepatitis and in chronic hepatitis such as presumed by HAMMARSTEN (1). But the abnormal heterogeneous curve appears for a long time in chronic hepatitis, whereas in cases of acute hepatitis, it only appears at the climax of the disease. HAMMARSTEN endow the form of the curve with prognostic value in cases of hepatitis. Fig. 31 shows that the curve is abnormal as long as the population is heterogeneous, and thereby indicates a disturbance in the erythropoiesis.

The conditions mentioned under point A—D show that the analysis of the curves reflects real conditions. Regularity, as shown

in fig. 31, and which according to table 46 appears in all patients, and a concordance with the theoretical distribution as good as that shown in fig. 30 can not be due only to chance.

Do the large blood cells originate in the bone marrow? Two other possibilities might be considered. Firstly, that the blood cells in this new group may be of normal size but may be coloured differently. If they were more polychromatic they would be more blue, and would then, according to experiments nos. 9—11, appear larger. But this possibility is excluded since all photographs have been taken with a yellow filter. The change in size caused by a difference in colour is approximately 0.3 my, whereas the difference between the two classes of blood cells is 0.75 to 1.00 my.

One might think that the size of the blood cells is normal, but that their surface tension has been reduced in such a way that they are flattened out during the spreading of the film, thereby showing a larger diameter (GÜNTHER (2)).

If this takes place, one might expect the pathological blood cells to be stretched out in the lengthwise direction of the blood film (experiment no. 16). 5 preparations, which according to the analysis should consist mainly of macrocytes, were measured along and across the direction of the film. The difference in MD observed in the two directions was  $0.192 \pm 0.07$  my, or very nearly the same difference which was observed when measuring the normal blood films. The obvious anisocytosis and the probable heterogeneity of the blood cells has further been reported by investigators who undertook the measurements in wet preparations where this error can not occur (C. GRAM, HAMMARSTEN & STÅHLE).

## Other differences between macrocytes and normocytes.

### *Mean thickness.*

In chapter VIII it is mentioned that the patients in this material had no increase in the corpuscular volume. But if the diameter increases without a simultaneous increase in the volume, then the thickness of the blood cells must decrease. The mean thick-



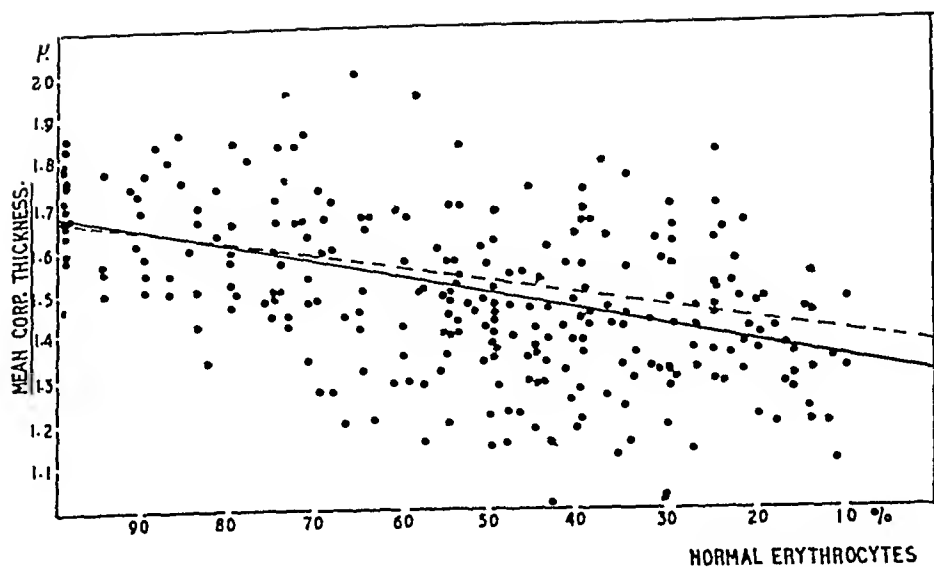


Fig. 32: Corresponding values for the mean corpuscular thickness and percentage of normocytes in cases of hepatitis. Stippled line: Expected decrease in MT. Drawn line: The correlation line of the diagram.

ness of the blood cells, MT, is usually calculated according to the formula:

$$MT = \frac{\text{corp. vol.}}{\frac{\pi MD^2}{4}}$$

regarding the blood cell as a cylinder, and disregarding that it is biconcave. MT is therefore dependent upon the accuracy in the determination of the mean diameter, the haematocrit determination, and upon the red cell count. Errors in all these determinations will add together and make the value of MT very uncertain. But in a large number of observations one might expect that the mean values would be comparatively accurate. The uncertainty of the determination will be expressed by a great variation around the mean. According to table 33 the mean corpuscular volume is 80 cub. my and MD 7.8 my in my material. The average value for MT should therefore in normal persons be 1.68 my.

The average diameter of the pathological large blood cells is 8.61 my. Since the volume is not increased, the corpuscular volume will still be 80 cub. my and the MT for these cells should therefore

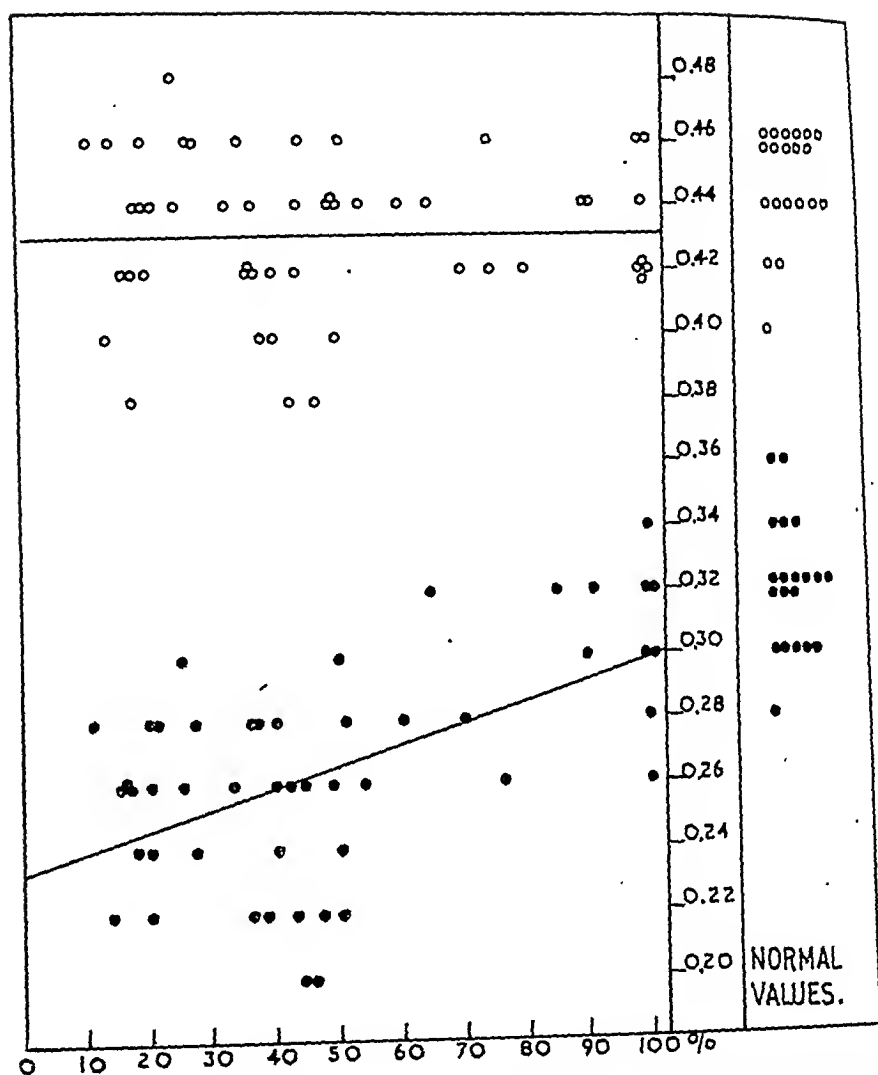


Fig. 33: Corresponding values for red cell fragility and percentage of normocytes in each sample. o : Beginning of haemolysis. • : Total haemolysis. (Normal values from table 33).

be 1.38 my. When, therefore, the number of pathological large blood cells increases from 0 % to 100 %, MT ought to decrease from 1.68 my to 1.38 my.

Figure 32 shows MT in my material of hepatitis distributed according to the number, in %, of normal erythrocytes in each sample. The stippled line represents the expected decrease in MT from 1.68 my to 1.38 my. The fully drawn line is the regression line of the diagram which shows a decrease as expected.

### *Red cell fragility.*

MEULENGRACHT, CHAUFFARD, GEIL and RIETTI mention that the red cell fragility increases in jaundice. DAVIDSON & GULLAND find the same in pernicious anaemia, and STEPHENS mentions that immature cells are less fragile than mature cells.

In my material red-cell fragility has been determined 49 times simultaneously with the determination of MD. In fig. 33 the values are marked in together with the percentage of normal blood cells in each sample. The diagram shows that the regression line for the beginning of haemolysis (marked with  $\circ$ ) is horizontal. There is, however, a definite correlation between the amount of normal blood cells in the sample and the value for total haemolysis (marked with  $\cdot$ ).

The explanation may be that there are always some normal blood cells in the blood from persons suffering from liver disease. These cells will have a normal fragility, and the haemolysis will therefore start at normal values. But the pathological, large blood cells have a higher resistance against hypotonic salt solutions, and the greater their number, the more difficult it is to obtain total haemolysis.

### **The regeneration of normocytes in acute hepatitis compared with regeneration of erythrocytes in pernicious anaemia.**

The main argument for the theory that the macrocytosis is caused by disturbances in the peripheral blood was that MD changed so rapidly that any other explanation was impossible (chapter IX, fig. 28). In this chapter it is shown that the increase in MD is caused by the appearance of a new type of blood cell. It is the number of these large blood cells, macrocytes, in each sample which mainly determines the size of the MD. In table 46 is given, in percentage, the contents of macrocytes in the individual samples, as given by the analysis. But when a patient is followed with examinations through a longer period, the total number of erythrocytes, and thereby also the number of macrocytes, may vary. We have therefore, in the following, calculated the

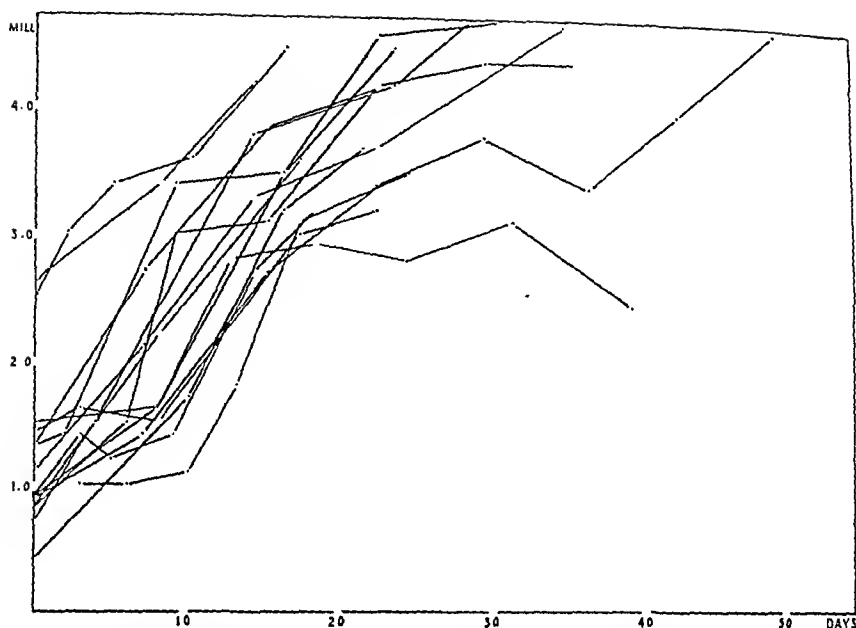


Fig. 34: Rise in number of normocytes during the time of observation. Acute hepatitis. Case 1—15.

number of normocytes in millions per  $\text{mm}^3$  for each sample, and these values are utilized when nothing else is mentioned.

72 observations in all, from the 15 patients with acute hepatitis who were observed during the whole course of the disease, are given in diagram fig. 34. These patients have the highest value for MD, and therefore the lowest number of normocytes, just after admission in hospital. From then on the number of normal blood cells increases with approximately the same speed in all patients, except in patient no. 15 who developed a considerable anaemia. The diagram further gives the impression, which also is confirmed by calculations, that the increase is not linear. The rate of increase is most rapid during the first period of the disease and then decreases gradually.

The diagram expresses how rapidly normal blood cells are added to the peripheral blood during the healing of acute hepatitis or, in other words, gives an expression for the regeneration of the normocytes. Does this regeneration in acute hepatitis occur with normal speed? In order to answer this question we need a material for comparison, where the rapidity of regeneration of the red blood cells is known. Such a material is available in un-

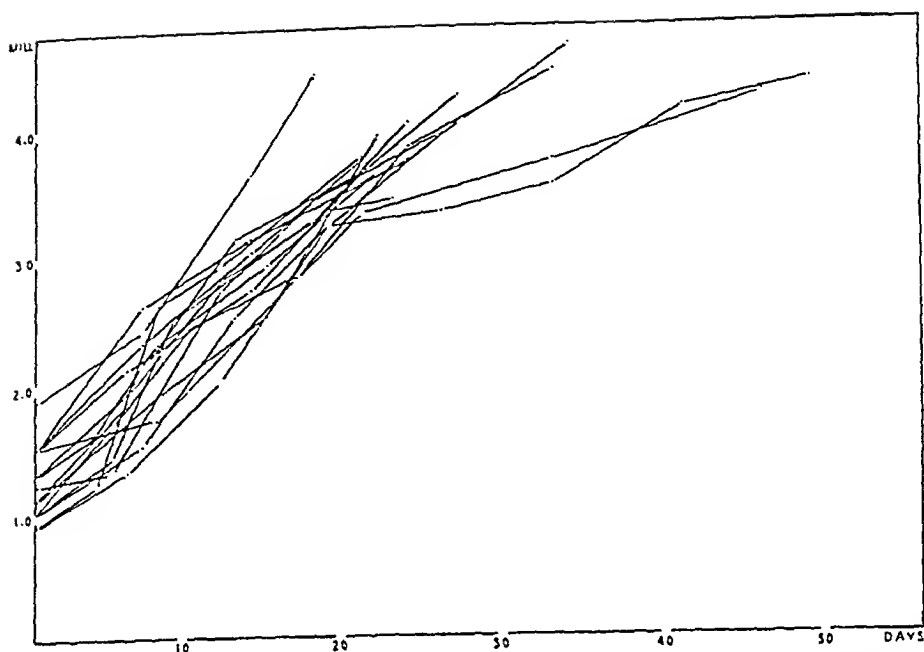


Fig. 35: Rise in number of erythrocytes in 15 cases of pernicious anaemia during specific treatment.

treated cases of pernicious anaemia. When these patients receive adequate treatment, the rapidity of regeneration is shown by the increase in the red blood cell count, and the beginning of the regeneration can be timed to the moment when the therapy starts. In the reticulocyte crisis we have a control for the efficiency of the treatment.

The patients who today are admitted to hospitals with pernicious anaemia often suffers from complicating diseases. Uncomplicated cases are usually treated as ambulatory cases. In order to obtain as pure a material as possible, patients who had been treated in the first years of liver therapy, from 1927 to 1932, were chosen. 15 patients were selected from the case reports of the University Hospital, Med. Dept. A from this period. It was emphasized that the disease should be quite typical, and that the therapy had a maximum effect when judged by the reticulocyte count. For this evaluation I used tables by RIDDLE and ISAACS & FRIEDMAN, given by HADEN (8). Furthermore, patients were chosen, whose sex, age, and initial blood cell count were as close to those of my patients as possible. We have thus made a selection of patients.

Daily blood counts were taken of these patients during the first period of treatment. During the later course of the disease the blood counts were less frequent. There will therefore be a greater number of counts

of the lower values than of the higher. This will, in the statistical treatment, «weigh» the curves when the regeneration is curvilinear. To avoid this, only approximately one blood count per week was used. The period of observation was from the beginning of the treatment until the maximum effect of the first liver doses was attained.

Fig. 35 indicates the increase in the number of red blood cells for these 15 patients, in the same way as for the cases of hepatitis in fig. 34. It is apparent that the increase in the total number of red cells in pernicious anaemia occurs with approximately the same speed as the increase of normocytes in acute hepatitis. Fig. 35 shows further, that the regeneration is not linear, but seemingly of the same type as that in hepatitis.

That the regeneration of red cells in pernicious anaemia is not linear is a well known fact. Since 1930 it has been known that the regeneration of erythrocytes in pernicious anaemia follows the formula for mono-molecular autocatalytic reactions, or the formula for biologic growth.

### The biologic growth formula.

ROBERTSON showed in 1923 that all growth which can be measured follows the equation which applies to autocatalytic mono-molecular reactions:

$$t-t_1 = K \log \left( \frac{x}{a-x} \right) \quad (1)$$

where:

$a$  is the limit value of the growth.

$x$  is the growth until the time  $t$ .

$t_1$  is the moment when  $x = \frac{1}{2} a$ .

$K$  is a constant, specific for each kind of growth.

This equation applies to the growth of bacteria in cultures, to the growth of humans, animals and plants, to the regeneration of damaged tissues, and to a number of other forms of growth (ref. WETZEL for a survey). RIDDLE showed in 1930 that the equation also applies to the regeneration of red blood cells in the treatment of pernicious anaemia, and it has later been shown that the

equation also applies to the regeneration of both blood cells and haemoglobin in other anaemias (SCHJØDT (2.4) H. C. GRAM (2) GJERD-SØ, STURGIS & ISAACS).  $K$  varies in each type of regeneration. In the case of pernicious anaemia, it was calculated by RIDDLE to be 18.5 days and by SCHJØDT to be 16 days.

In order to apply the formula to the present material, we first have to find  $K$ . It is then convenient to write equation (1) in a slightly different way:

$$\log \left( \frac{x}{a-x} \right) = K' (t-t_1) \quad (2)$$

taking the time as the independent variable.

Equation (2) is the equation of a straight line of the form

$$y = K' x$$

The average value for the red blood cell count in my material of acute hepatitis is 4.6 mill. pr. mm<sup>3</sup>. We therefore choose  $a = 4.6$  mill. and  $\frac{1}{2}a = 2.3$  mill., which is taken as the zero point on the Y-axis.

To determine  $t_1$  we take advantage of the fact, shown in fig. 34—35, that the increase in the number of red cells seems to be linear in the central part of the curves. For each of the 15 patients with acute hepatitis, and for each of the 15 patients with pernicious anaemia, the linear regression for the data in fig. 34 and fig. 35 was calculated. The day when the number of cells was 2.3 mill. might then be found, and this day was in each case taken as  $t_1$ . The

linear regression for corresponding values of  $(t-t_1)$  and  $\log \left( \frac{x}{a-x} \right)$

according to equation (2) was then calculated. Finally it was controlled graphically that the regression line passed through the point  $t_1/2.3$  mill. The regression coefficient found in the last set of calculations is an expression of  $K'$ .

It was now found that the regression coefficients for the 15 cases of acute hepatitis and for the 15 cases of pernicious anaemia were nearly identical, both concerning the linear regression and concerning the logarithmic curve in equation (2).

For the linear regression, the coefficients were:

For acute hepatitis  $0.122 \pm 0.038$

For pernicious anaemia  $0.110 \pm 0.024$

and for the logarithmic curves (equation (2).

For acute hepatitis  $0.063 \pm 0.018$

For pernicious anaemia  $0.049 \pm 0.013$

In neither of the two series is there a significant difference between the value obtained for acute hepatitis or pernicious anaemia, the variance ratio being respectively 1.07 and 2.36\*. Accordingly, it seems reasonable to assume that the speed of regeneration is the same both when it concerns the formation of normocytes in acute hepatitis and the formation of red blood cells in pernicious anaemia.

The regression coefficient for the logarithmic curves in equation (2) is a measure of  $K'$ . As  $K' = \frac{1}{K}$  it follows that  $K$  in equation (1) will average 15.8 days in the cases of acute hepatitis, and 20.6 days in the cases of pernicious anaemia, or very close to the value previously found by RIDDLE (18.5 days).

*Is the regeneration of normocytes adequately expressed  
by the biologic growth formula?*

In order to answer this question all observed values in fig. 34, in all 72 observations, were grouped in classes, with class-intervals of one day, reckoned from  $t_1 = 0$ . The total variance of this values may be divided into one part due to the regression of the number of cells on time, a part expressing the deviation from the regression line, and a residual. This last residual expresses the variance within each class. If the variance between classes, after regression on time is eliminated, differs significantly from the variance within classes, then the regression line found does not give a correct expression for the regression of number of blood cells on time (cfr. BONNIER & TEDIN pg. 190).



TABLE 39.  
*Analysis of variance of data in fig. 34 and 35.*

Item	Degrees of freedom	Variance	Variance ratio
<i>The linear regression:</i>			
Regression . . . . .	1	94.211	} 3.70***
Deviation from regression .	36	0.318	
Residual . . . . .	34	0.086	
Total . . . . .	71	1.529	
<i>The biologic growth formula:</i>			
Regression . . . . .	1	25.693	} 1.45
Deviation from regression .	36	0.084	
Residual . . . . .	34	0.058	
Total . . . . .	71	0.432	

The present data were analysed accordingly (table 39).

In the case of the equation for the linear regression the variance ratio is significant. The analysis thus shows that the increase in normocytes in acute hepatitis is certainly not linear.

In the case of the logarithmic curve for biological growth the corresponding variance ratio is not significant. *The conclusion must be that ROBERTSON's formula for growth, with  $K = 15.8$  days, in a satisfactory way expresses the rise in normocytes in acute hepatitis.*

This is also shown in fig. 36 where the growth curves calculated according to equation (1) are drawn. The values observed from fig. 34 and 35 are marked in, and fall, as expected, very nicely around the curve.

This analysis shows the rate of production of normocytes during the recovery period of acute hepatitis. The reverse condition, the decrease in normocytes and production of macrocytes during the development of the disease, can not be shown by means of the material at hand. The greater number of patients had the highest observed value for MD, and consequently the largest proportion

of macrocytes shortly after admission. The appearance of macrocytosis must therefore have occurred prior to admission to hospital.

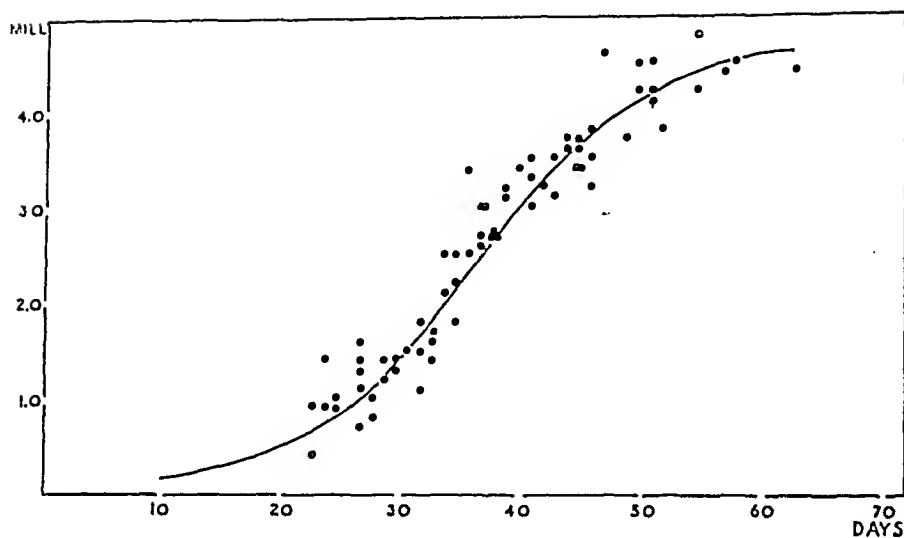
The results of the analysis of the frequency curves, together with the arguments put forward pg. 128—137, point definitely to the cause of the macrocytosis being a changed erythropoiesis, and not any condition in the peripheral blood.

The rise in MD will be determined by the number of pathological, large, blood cells which at any given moment circulate in the blood stream. But the speed with which these pathological cells are produced by the bone marrow, and are passed into the blood-stream, will be determined by the rate of destruction of the already circulating cells. As these patients do not have any severe anaemia, and as there is no indication of increased cell production, one may assume that the destruction of the already circulating cells occurs with normal speed.

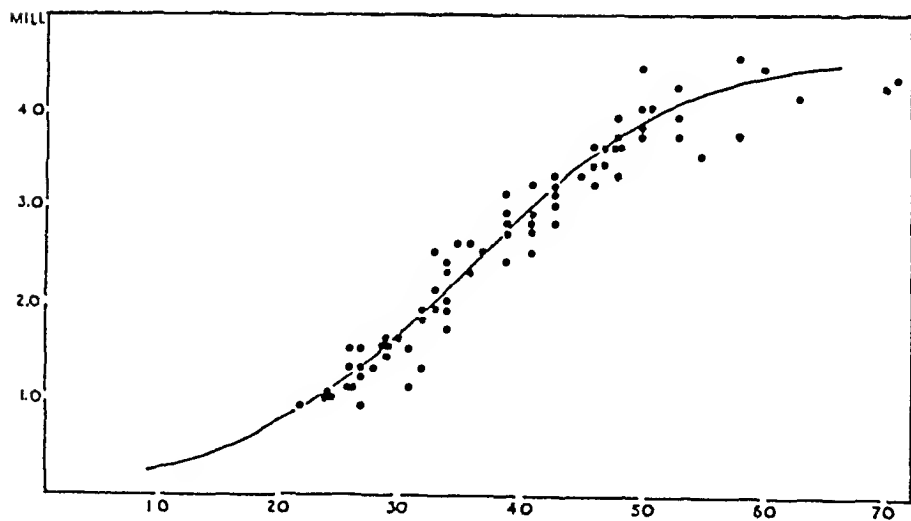
In acute hepatitis, the value for MD goes from the highest observed values to normal values in approximately 20 days (see table 46 and fig. 28). The time interval from the day when, according to the case-reports, the patients observed the first symptoms of the disease, until the maximum value for MD is observed, average 17 days. Considering the inaccuracy of the information given by patients, and considering that the measurements have been done approximately once a week, this must be considered a satisfactory concordance. I therefore presume that the macrocytes appear and disappear in the peripheral blood with approximately the same speed. But for this to be true, it is also necessary to assume that the development of macrocytosis may start prior to jaundice being observed by the patient.

It has thus been shown that the decrease in MD observed in acute hepatitis is due to production of normocytes which replace the pathological large blood cells circulating in the blood at the height of the disease. This production of normocytes occurs with the same speed as that with which erythrocytes are produced in pernicious anaemia treated with liver extract. Probably, but not certainly, the macrocytes are produced with the same speed. But then the main argument for the theory of the peripheral cause of the macrocytosis in liver diseases is cancelled. For there can be no doubt that the changes seen in pernicious anaemia is caused by a changed erythropoiesis.

Fig. 36: The biologic growth formula.



Acute hepatitis. Case 1—15. Rise in number of normocytes during the time of observation.



Pernicious anaemia. 15 cases. Rise in number of erythrocytes during specific treatment.

*The life-time of the red blood cells.*

The life-time of the red blood cells has been determined by different methods. The results vary from 30 days (DEKKERS) 60—90 days (SCHJØDT) to about 120 days (CALLENDER & NICKEL, WINTROBE, WHITBY & BRITTON, OWREN, SCHRUMPF) according to the method used. The last value is probably correct. In certain pathological conditions, particularly in haemolytic jaundice, the life-time is considerably shortened, to approximately 4 to 6 days (KIERKEGAARD & KIERKEGAARD) or 10—12 days (OWREN). But in such cases one always has signs of an increased regeneration as reticulocytosis, polychromasia and appearance of nucleated red blood cells in the peripheral blood.

SCHJØDT has also attempted to determine the life-time of the blood cells by means of the biological equation of growth. If the formula:

$$t - t_1 = 15.8 \log \left( \frac{x}{a - x} \right)$$

is used as a basis,  $x$  will increase from 0.1 to 5 millions during 55—60 days. But it is hardly justifiable to use the formula in this way. The formula shows that the regeneration speed decreases when the values approach normal values. Therefore, where the blood cell count is normal or very nearly normal, the biological equation of growth will not give a correct expression of the regeneration. The 55—60 days, which is calculated from the equation, is only the *maximum* regeneration speed possible in pernicious anaemia and in liver diseases. It will later be shown (chapter XII) that in the present material the regeneration never surpasses this maximum.

### Résumé of Chapter X.

Based on the results in chapter IX, the working hypothesis that the blood cell population in hepatitis is heterogeneous and consists of two components is introduced. By analysis of the frequency curves, according to the method described in Chapter VI, it is shown that the blood cell populations in liver diseases consist of two distinct components: One component of normal

erythrocytes with MD: 7.8 my, and one component consisting of blood cells with an average diameter of 8.6 my. These large blood cells are thinner than the normal erythrocytes, and are more resistant against hypotonic salt solutions.

It is shown that the pathological frequency curves have a satisfactory concordance with the corresponding theoretical curves when measured by  $\chi^2$  analysis. The relative strength of the two components varies in a regular way. The regeneration speed of the normocytes in acute hepatitis compared with the regeneration speed of erythrocytes in pernicious anaemia is in both cases the same. It is shown that a very good expression for the regeneration in both cases is a curve expressed by the equation for monomolecular autocatalytic reactions, also called the equation for biological growth, as given by ROBERTSON in 1923.

## CHAPTER XI.

### The red blood cell diameter in pernicious anaemia.

The population consists of at least three components.

In the introduction it was mentioned that a number of authors were of the opinion that the macrocytosis in liver diseases is identical with that observed in pernicious anaemia.

But there are still considerable differences between the two conditions. In chapter VIII it is shown that the macrocytosis in liver diseases never is accompanied by an increase in the corpuscular volume. In pernicious anaemia, the corpuscular volume is regularly increased beyond the upper normal limit. (HADEN, OSGOOD, HASKIN & TROTMAN, WINTROBE).

Fig. 13, page 83, shows that the curves in cases of untreated pernicious anaemia have a completely different appearance compared with the curves from patients with liver diseases. In pernicious anaemia the curves are considerably broader, with a much higher value for  $s$ . This has previously been shown by PRICE JONES, MOGENSEN, DALAND, HEATH & MINOT and others. In this respect pernicious anaemia differs from all other diseases.

In fig. 37, left, 5 frequency curves are given from patients with untreated pernicious anaemia. To the right are given the frequency curves from the same patients when in partial remission, 27—50 days after the treatment had commenced, and 14—30 days after the finished reticulocyte-crisis. These curves were analysed in the same way as described before.

$m_p$  was determined both according to my method, and according to MOGENSEN's method, and gave in most cases concordant results.

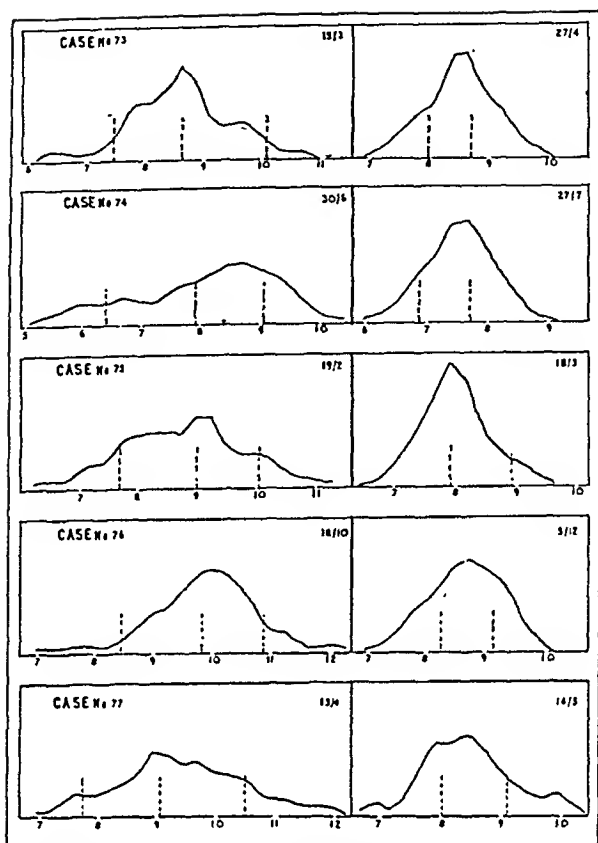


Fig. 37: Frequency curves from five cases of pernicious anaemia.  
Left: Untreated. Right: In partial remission.

Wherever a difference in the value of  $m_0$  appeared, the value obtained by my method was used. The result of the curve-analysis, together with the results from 5 additional patients, are given in table 40.

When one compares the curves from untreated pernicious anaemia in fig. 13 and fig. 37, with curves from patients with hepatitis, as shown in fig. 31, the two sets of curves seem to be entirely different. But when the patients with pernicious anaemia are in partial remission, their frequency curves, as shown by fig. 37, can no longer by mere inspection be distinguished from the curves observed in hepatitis. This is also borne out by the analysis of the curves (table 41). Whereas the frequency curves in hepatitis can be divided only into two components, the curves from cases of untreated pernicious anaemia consist of at least three components.

TABLE 40.

*Pernicious anaemia. Untreated.*

Case no.	MD	s	m <sub>1</sub>	s <sub>1</sub>	N <sub>1</sub> %	m <sub>0</sub>	s <sub>0</sub>	N <sub>0</sub> %	m <sub>r</sub>	s <sub>r</sub>	N <sub>r</sub> %	m <sub>0</sub> ÷ m <sub>1</sub>	m <sub>r</sub> ÷ m <sub>0</sub>
73	8.63	0.79	7.50	0.50	14.5	8.88	0.47	73.0	10.00	0.41	15.5	1.38	1.12
74	8.02	1.15	6.29	0.46	16.0	7.88	0.60	38.6	9.00	0.52	46.0	1.59	1.12
75	8.63	0.91	7.60	0.47	27.2	8.88	0.58	66.0	9.93	0.57	10.0	1.28	1.05
76	8.84	0.81	7.39	0.57	9.0	8.85	0.52	70.0	9.75	0.54	11.2	1.36	1.00
77	9.41	1.03	7.71	0.33	11.0	9.00	0.50	49.6	10.40	0.63	39.2	1.29	1.40
78	9.13	0.93	7.90	0.55	21.2	9.25	0.51	68.0	10.71	0.55	11.3	1.35	1.46
79	8.15	1.02	6.69	0.58	16.2	8.00	0.54	57.5	9.34	0.55	28.0	1.31	1.34
80	8.65	0.97	6.97	0.47	9.0	8.38	0.56	64.0	9.78	0.50	28.5	1.41	1.40
81	8.87	1.01	7.93	0.81	36.5	9.31	0.57	59.5	10.78	0.35	4.5	1.38	1.47
82	8.81	1.16	7.81	0.77	46.0	9.50	0.50	52.5	10.90	0.32	7.3	1.69	1.40
Mean	8.71	0.98	7.38	0.55	20.7	8.78	0.55	59.9	10.06	0.49	20.2	1.40	1.28
<i>Mogensen, mean values.</i>													
	8.03	0.98	5.84		8.8	8.18	0.67	89.3	10.25		2.8	2.34	2.07
<i>Mogensen, mean values corrected (+ 0.60 my).</i>													
	8.63		6.44			8.78			10.85				
<i>Tollermann, mean values.</i>													
	8.42	0.88	7.23	0.76	18.3	8.67	0.68	81.7				1.44	
<i>Tollermann, mean values corrected (+ 0.30 my).</i>													
	8.72		7.53			8.97							



TABLE 41.

*Pernicious anaemia. Partial remission.*

Case no.	MD	s	m <sub>1</sub>	s <sub>1</sub>	N <sub>1</sub> %	m <sub>0</sub>	s <sub>0</sub>	N <sub>0</sub> %	m <sub>r</sub>	s <sub>r</sub>	N <sub>r</sub>	m <sub>0</sub> ÷ m <sub>1</sub>	Treatment Days
73	8.50	0.59	7.99	0.47	32.0	8.75	0.49	68.0				0.76	42
74	7.51	0.58	6.87	0.46	20.0	7.70	0.49	80.0				0.83	28
75	7.96	0.55	7.88	0.50	92.0	8.92	0.38	8.0				1.04	27
76	8.66	0.62	8.19	0.47	46.0	9.05	0.41	54.0				0.96	50
77	8.48	0.79	8.00	0.46	52.0	9.08	0.49	46.0				1.08	31
Mean	8.14	0.63	7.79	0.47	48.4	8.70	0.45	51.2				0.93	
<i>Mogensen, mean values.</i>													
	7.76	0.66	5.45			7.79	0.58					2.34	
<i>Mogensen, mean values corrected (+ 0.60 my).</i>													
	8.36		6.05			8.39							

In the available literature, I have found two authors who discuss the analysis of frequency curves. MOGENSEN has carried out the analysis in 16 cases of untreated pernicious anaemia, and in 7 cases of pernicious anaemia in partial remission. TØTTERMANN has carried out similar analysis in 5 cases of bothriocephalus-anaemia. The mean values of these two authors are also given in table 40. MOGENSEN utilizes Price Jones' original method and his normal value for MD is 7.2 my. In order to obtain comparable values his values for MD must therefore be adjusted by adding 0.60 my (see chapter V). TØTTERMANN has a normal value: 7.486 my, and his values for MD must therefore be increased by 0.30 my in order to be compared with my values.

TØTTERMANN utilizes a slightly different technique, and his method for analysing the curves is also somewhat different. His results may, therefore, only to a certain extent be compared with the results of MOGENSEN and the author.

A comparison of MOGENSEN's results and those of the author gives rise to important conclusions:

*The central component in pernicious anaemia* has in my material an average mean: 8.78 my. MOGENSEN has the same value after the correction has been applied. This value lies very close to the one found for the right hand component in acute hepatitis, 8.65 my. Whereas this component in the cases of hepatitis always has a normal standard deviation,  $s$  is significantly increased in 9 of my 10 cases of pernicious anaemia. The mean diameter of this component does not change when the patients receive specific treatment, but  $s$  decreases to normal values. There is no statistical difference between MOGENSEN's and my values for  $m_0$  in untreated pernicious anaemia (variance ratio 6.09) or in pernicious anaemia in partial remission (variance ratio 1.82). Neither is there any difference between  $m_0$  in my untreated and treated cases (variance ratio 2.24\*) or between  $m_0$  in my cases of untreated pernicious anaemia and my values for  $m_T$  in hepatitis (variance ratio 3.27).

*The left hand component in pernicious anaemia* has in my material an average mean: 7.38 my. MOGENSEN's value is considerably lower. This is partly due to the fact that we use different methods for the determination of  $m_0$ , and partly because MOGENSEN in

the curve analysis introduces the demand that «the main component must be as extensive as possible». I have not been able to accept this demand, because I am of the opinion that one thereby introduce an unjustified constraint in the method. But this leads to the result that MOGENSEN's main component must be larger, and that his secondary components must contain fewer frequencies than my secondary components.

My value for  $m$  in this component, 7.38 my, is definitely lower than the corresponding left hand, and normal, component in acute hepatitis which was 7.93 my. The variance ratio between this group and my normal values from table 33 is 7.19\*\*, which is significant. The left hand component in untreated pernicious anaemia therefore contains blood cells with a smaller diameter than normal. This may also be directly observed from the frequency curves: 6 of the 10 curves which were investigated have a number of frequencies in the classes 6 my and lower, and with my normal values this should occur only 4 times per 1000 blood cells. I therefore agree with MOGENSEN when he points out as a characteristic feature in pernicious anaemia that microcytes occur in the peripheral blood. When these patients are treated this component changes in character: The mean diameter of this component increases, in my material to  $7.79 \pm 0.52$  my, and can no longer be distinguished from the MD of normal blood cells (variance ratio 2.77). The analysis of variance for the means of this component between untreated patients and patients in partial remission gives a ratio of 26.94\*\*\*, which also shows that there is a definite difference. This can only mean that the pathological small blood cells, the microcytes, have disappeared and that the normocytes are the only ones that remain.

*The right hand component in pernicious anaemia* has in my material an average mean : 10.06 my. MOGENSEN finds somewhat higher values, due to difference in technique. These large blood cells are specific for pernicious anaemia, and have no counterpart in any of the hepatitis curves. They are probably identical with the megalocytes mentioned by earlier authors (SCHILLING, NAEGELI).

This group of cells disappears from the blood when the patients receive treatment. The mean corpuscular volume, which has been

increased, attains at the same time normal values. This happens simultaneously with the normalization of the bone marrow, with the disappearance of the megaloblasts, and simultaneously with, or immediately after, the reticulocyte-crisis (MOGENSEN).

As previously mentioned, can TÖTTERMANN's values for the characteristics of the individual components not be directly compared with MOGENSEN's and my own data. But his curves may also be split into two components, and they conform best with the curves found in pernicious anaemia in partial remission. He does not find any cells which correspond to the right hand component with particularly large blood cells, found in untreated pernicious anaemia.

**The blood cell population seen in pernicious anaemia is completely different from that seen in liver disease.**

In the peripheral blood of untreated pernicious anaemia there thus occur three types of abnormal cells:

A greater part of the blood cells have an diameter approximately 0.99 my larger than the diameter of the normal blood cells. Another group has a diameter of approximately 10—12 my, and probably increased corpuseular volume as well. These cells are probably identical with the megalocytes. Finally one finds a group of microcytes with a smaller diameter than the normal blood cells. The analysis has not directly shown that normal blood cells also are present, but it is reasonable to assume that they are, and that they are hidden in the left hand component of the curve.

Simultaneously with these haematological changes, we have the other changes in pernicious anaemia: The anaemia, the bilirubinaemia, the typical changes in the bone marrow, and the clinical symptoms.

When these patients are treated, the bone marrow becomes normal and the anaemia is healed. The megalocytes and the microcytes disappear. *But the macrocytosis remains, in spite of specific treatment.* MD remains increased, and this symptom does not disappear (SCHULTEN (1), MOGENSEN (1), KIRK (2, 3). My material does not permit any substantiation of this assertion. But MOGEN-

SEN has a group of 14 patients with pernicious anaemia in «maintenance treatment» with a normal red cell count. These 14 patients have an average mean diameter of  $7.306 \pm 0.190$  my, against his normal mean diameter,  $7.152 \pm 0.146$  my. The variance ratio between the two groups is 51.14\*\*\* which is significant. The measurements in these 14 patients were carried out 123 to 702 days, on an average 329 days, after the commencement of the treatment. The average increase in MD compared with the normal value is 0.154 my. According to table 30 this corresponds to about 20 % macrocytes in the peripheral blood.

Table 41 shows that this increase in the mean diameter in treated pernicious anaemia is due to macrocytes. These macrocytes have a normal corpuscular volume and must therefore be thinner than the normal blood cells (see page 147). DAVIDSON & GULLAND, and RIETTI have shown that red cell fragility also is increased in pernicious anaemia, as in the case of the macrocytes in hepatitis. These cells can not be distinguished from pathological blood cells seen in hepatitis by any criteria utilized in this work, and I am of the opinion that they are of the same type.

Since these cells remain in spite of the specific treatment of the pernicious anaemia, their presence can not be due to any lack of the «anti-pernicious principle». I agree with SCHULTEN and SCHULTEN & MALAMOS who maintain that the blood cells in hepatitis are identical with cells observed in pernicious anaemia in remission. But the changes are not caused by any lack of «intrinsic factor».

The erythropoiesis in pernicious anaemia is thus changed in two different ways. Firstly, the specific changes which are mentioned above and which disappear with adequate treatment. These changes are typical and characteristic of pernicious anaemia.

Secondly, those changes which remain after the treatment has brought about full remission of the anaemia. These changes cannot be separated from those observed in hepatitis, and I believe that this disturbance of the erythropoiesis has the same cause in pernicious anaemia as in the other diseases where macrocytosis occurs, such as in liver diseases, in serious haemorrhage (SJÖWALL), in tropical anaemia (GOODHART), in bothriocephalus anaemia (TOTTERMANN) and in other conditions.

This macrocytosis may appear without simultaneous serious anaemia, as in the present material. But it is still an expression for a disturbance in the erythropoiesis.

### Résumé of Chapter XI.

In this chapter the macrocytosis in liver diseases is compared with the macrocytosis in pernicious anaemia. It is shown that whereas the blood cell population in hepatitis consists of 2 components, in untreated pernicious anaemia it consists of at least 3, probably 4 components: One main component which corresponds to the macrocytes observed in liver diseases. One component with considerably larger cells, probably identical with the earlier authors' megalocytes. One component consisting of microcytes as previously shown by MOGENSEN. And finally, probably one component with normal blood cells which I, however, have been unable to demonstrate directly. When the patients are given the antipernicious principle the microcytes and megalocytes disappear rapidly. But the macrocytes remain for years, in spite of adequate treatment. The frequency curves found in pernicious anaemia in partial remission can not be distinguished from those seen in liver diseases by any criteria which are utilized in this work.

The conclusion must be that this macrocytosis cannot be due to any lack in the anti-pernicious principle, but must have another cause.

## CHAPTER XII.

### Final discussion.

In chapter 1, page 25, the problems which remain unsolved after a survey of the literature are stated in the form of 5 questions. Some of these questions may now be answered by means of the results of the investigations reported in chapters VIII—XI.

**1) When does macrocytosis appear in liver diseases? How fast does the phenomenon appear and disappear?**

The present investigation gives the same results as previously reported by a number of authors: A constant increase in the mean diameter, without a simultaneous increase in the cell volume, is seen in all cases of hepatitis. In hepatitis there is therefore a «macroplania» of the blood cells, as previously pointed out by ARCH, SCHULTEN and DEDICHEN. This fact explains the discrepancy between the authors who have determined the mean diameter of the red cells, and the authors who have determined the mean corpuscular volume. In patients with obstructive jaundice, the size of the cells is normal.

The macrocytosis persists during the whole course of the disease, provided the patient does not receive any special treatment (see later).

When the patient recovers, the macrocytosis disappears, and MD becomes normal. The change in MD average in my material 0.014 my per day, which agrees well with observations by MOGENSEN and C. GRAM.

## 2) Is the macrocytosis due to changes in the peripheral blood, or is the cause a changed erythropoiesis?

In chapter IX it is definitely shown that none of the changes in the peripheral blood with which earlier authors have tried to explain the phenomenon, can be the cause. In chapter X it is shown that the macrocytosis is due to a changed erythropoiesis: Macrocytes, pathological large cells, with an average increase in diameter: 0.85 my, are produced, and the blood cell population in the peripheral blood becomes heterogeneous. The altered mean diameter of the blood sample is only an expression of the number of macrocytes present in the peripheral blood. Since the blood cell population becomes heterogeneous, the distribution curves of the red cell diameters will be broader than normal, with increased standard deviation. These findings explain the clinical observations made by HAMMARSTEN, that an increased standard deviation of the frequency curve indicates that the liver disease is still active. When the patient recovers, the macrocytes are substituted by normocytes. The production of normocytes occurs in concordance with the biological growth formula, and at approximately the same rate as that with which erythrocytes are produced in pernicious anaemia during treatment. It seems reasonable to assume that the production of macrocytes in the beginning of the disease occurs at the same rate.

## 3) Has the macrocytosis in liver disease the same cause as the macrocytosis in pernicious anaemia?

In chapter XI it is shown that macrocytosis in liver diseases is not identical with macrocytosis observed in untreated pernicious anaemia. In untreated pernicious anaemia we find megalocytes and microcytes, two cell forms which are not observed in liver diseases. But we also find macrocytes, and these macrocytes cannot be distinguished from those seen in liver diseases by any of the criteria which are utilized in this work. When patients with pernicious anaemia receive adequate treatment the cells which are typical for this disease disappear. But the macrocytosis remains in spite of the treatment. According to calculations based on MOGENSEN's investigations this macrocytosis re-



mains for months and years after the specific treatment has commenced, and in spite of full remission of the haemoglobin and the number of red blood cells.

This, together with the arguments which are given in the introduction (page 20), makes it unlikely that the macrocytosis in liver diseases is caused by any lack in the antipernicious principle, as usually assumed (see page 22).

#### 4) If the macrocytosis in liver disease and in pernicious anaemia is not due to the same cause, what is then the cause of this phenomenon?

This question cannot be answered by the previous investigations. But these investigations may possibly indicate how a solution may be attempted.

#### *The normal erythropoiesis.*

The normal erythropoiesis demands that two processes must follow a normal course: Firstly, a sufficient number of erythrocytes must be produced in the bone marrow, to replace those blood cells which perish in the peripheral circulation. Secondly, the haemoglobin synthesis must be normal, so that these newly formed blood cells become saturated with haemoglobin.

CAPPS, already in 1903, showed that we here have two distinct processes, which do not necessarily follow a parallel course. And this has later been confirmed by a number of authors: By SAWALL in bleeding experiments, by WHIPPLE, ROBSCHT-ROBBINS and others in feeding experiments, and by HAWKINS, HAUN & others in experiments with radio-active iron. SCHMIDT, and later GRENDS, have shown that the synthesis of haemoglobin also occurs in concordance with the equation for mono-molecular auto-catalytic reactions, but with another value for  $K$  than the one which applies to the production of the cells. And finally, THORELL has recently, by analysis of the single cells, shown that the haemoglobin synthesis is an independent process.

The formation of the erythrocytes takes place in the bone marrow from a relatively small number of parent cells. The formation depends upon two distinct processes, growth and maturation

of the cells. The growth, i. e. the formation of a sufficient number of cells, is connected with the youngest precursors of the blood cells. Already the number of the different cells in the bone marrow seems to indicate this: 2—6 % of young immature proerythroblasts gives rise to 35—40 % basophile normoblasts and to 50—60 % orthochromatic normoblasts (WHITBY & BRITTON). Some haematologists (ROHR, KIENLE, HABELMAN) are opposed to this view, but THORELL, in the above mentioned work, has shown that the new-formation of the cells (the growth proper) takes place at the earliest stages of development, and that it is normally finished, or nearly finished, when the maturation starts.

The maturation of the cells, or the development from the younger forms to the mature erythrocyte, also takes place in the bone marrow. There is some disagreement as to whether this development from the immature to the mature cell is a continuous process (WHITBY & BRITTON, HADEN (8), SCHILLING, NAEGELI), or if the process is discontinuous. This is maintained by KIENLE (mitosis studies), FREERKSEN (comparative anatomical studies) and by FREERKSEN, ALDER and ROHR, based on clinical observations and morphological studies.

If the *haemoglobin synthesis* is reduced, while the cell production remains normal, hypochromic anaemia develops.

If the *growth* of the cells is restricted, we get hypoplastic, respectively aplastic anaemias.

If the *maturation process* is disturbed, we obtain changes of the kind which are discussed in the present work.

### *Disturbed maturation of the blood cells.*

There is, as mentioned, no agreement whether the maturation is a continuous or a discontinuous process: Whether one cell, as for instance a pro-erythroblast, may divide into two proerythroblasts (homoplastic division) or into two macroblasts (heteroplastic division).

Without taking any side in this controversial question, one must, however, be allowed to maintain that the development of the cells passes through one or more «critical stages». In order

to bring the development of the cells through these stages certain substances or principles, only partly known, are necessary.

The proerythroblast represents one such «critical stage». The development of the cells will be completely or partly arrested here if there is a lack in the «anti-pernicious principle». The growth will continue as before (THORELL) and the bone marrow presents the picture typical for untreated pernicious anaemia — a marrow increased in volume and invading the fatty marrow. The greatly increased number of cellular elements mainly consist of the younger forms of the erythropoietic cells, the proerythroblasts. Simultaneously, an absolute and relative reduction in the number of normoblasts occurs. The proerythroblasts mature into megaloblasts, cells with an abnormal pyenotic nucleus, partly or wholly filled with haemoglobin. These megaloblasts probably give rise to the megalocytes in the peripheral blood. A salient feature is that these cells are not reduced in size during maturation, in contrast to erythrocytes formed in normal marrow. As an expression for a materially reduced maturation and production of erythrocytes, anaemia develops.

When these patients receive the «anti-pernicious principle» the maturation continues. Already the day after the treatment has started, the marrow is seemingly normoblastic and apparently normal blood cells are streaming into the blood. The whole picture is what we might expect if a blockade had been lifted.

But the erythropoiesis is still not normal. The «normoblasts» of the bone marrow are still larger than normal (NORDENSON (4) and this macroblastic marrow remains long after the reticulocyte crisis is finished. (NORDENSON (4), SCHULTEN (3). At the same time macrocytes circulate in the peripheral blood, and this macrocytosis may persist for months and years after the treatment has been started, and long after the blood counts have returned to normal values (KIRK (2), MOGENSEN (1), SCHULTEN (1) (see also page 167). It is reasonable to see these two conditions as related, and to assume that the macrocytes in the peripheral blood originate from the macroblasts in the bone marrow.

The similarity between the changes seen in the bone marrow in pernicious anaemia and in liver diseases makes it probable that the macrocytosis as well, is due to a deficient maturation, but at a later stage in the development than the megaloblastic stage.

Even in macrocytosis we find an increase in the number of the younger erythropoietic cells, and an increase in the volume of the red bone marrow. (ROSSIER, BLEICHROEDER). The increased number of erythropoietic cells is mainly due to macroblasts (ISAACS, ALDER), as if the growth and the development progressed normally until this stage. These macroblasts seem to be more easily developed into macrocytes than the megaloblasts are developed into megalocytes, since we do not obtain the same grave anaemias as observed in pernicious anaemia. But a moderate degree of anaemia is observed here as well (see page 120). In liver diseases the number of macroblasts in the bone marrow is greater than normal. When the blockade is lifted the maturation progresses, and normoblasts with subsequent formation of normocytes are formed. The production of normocytes then occurs at the same rate as the production of erythrocytes in pernicious anaemia during specific treatment. This also confirms that the two phenomena in principle are of the same nature. Both in pernicious anaemia and in liver diseases, we are faced by a deficient maturation.

### *The cause of deficient maturation.*

CASTLE & MINOT have shown that the megaloblastic bone marrow in pernicious anaemia is due to a lack in «the antipernicious principle». We now know that this principle is not one single substance, but consists of a series of components where each component has its specific action (DEDICHEN, BJØRNSON, BARFRED). For some time after the discovery of folic acid one believed that one had found the specific substance, necessary to mature the proerythroblast into the macroblast, and that a deficiency in this substance leads to the development of megaloblasts. More recent researches (HEINLE, DINGLE & WEISBERGER, VILTER, VILTER & SPIES, HADEN (9) and others), seem to indicate that a lack of folic acid is not always the cause of deficient maturation. It is more probable that folic acid exists in the food in conjugated form, and that we need another principle to release the folic acid so that it may be utilized in the organism.

Folic acid belongs to the vitamin-B group and it is probable that other members of this group also take a part in the erythropoiesis.

Thus GOODHART, VAUGHAN (1) and WILLS mention that they have seen improvement in macrocytic anaemias after administration of yeast and manitt and it has also been shown in animal experiments that a series of the Vitamins B are of importance (see survey by WINTROBE, 7, page 97).

CAYER, RAFFIN & PERLZWEIG have shown that the amount of niacin, riboflavin and thiamin is sub-normal in patients with pernicious anaemia and AHLSTRÖM has found sub-normal values for vitamin-B in patients with liver cirrhosis. VILLA and BEIGLBOCK & SPIESS-BERTSCHINGER finally report that liver diseases and the accompanying anaemias improve by the administration of large doses of niacin.

These observations may possibly indicate that the cause of the deficient maturation is due to a lack in one of the members of the vitamin-B group.

### *Does a lack of vitamin-B play a role?*

To answer this question the present material of chronic hepatitis may be used. These patients have, as shown in table 46, a macrocytosis which persists through weeks and months. If the reason is that these patients lack a certain principle, then one might expect that the macrocytosis would disappear when this principle was supplied. The maturation should become normal, and normocytes ought to appear in the peripheral blood at a rate which may be predicted by the equation for biologic growth (see page 152—156).

If this hypothetical principle was again withdrawn, one might expect that the macrocytosis in the peripheral blood would reappear, and again at a rate which may be predicted by the formula.

The author has carried out some experiments to test this hypothesis:

Fig. 38 shows the number of normocytes in millions per  $\text{mm}^3$  in 4 patients with chronic hepatitis who did not receive any treatment. The curves show that the number of normocytes remains fairly uniform through several months.

Fig. 39 shows similar curves from 4 patients with spontaneous improvement in their chronic hepatitis. The number of normo-

cytes increases to some extent, and the agreement with the biological equation of growth is in all cases fairly good.

Fig. 40 shows the curves of 3 patients who received liver extract as used in pernicious anaemia without an increase in the number of the normocytes.

Fig. 41 shows the curves from 2 patients who received folic acid without any effect.

Fig. 42 shows the curves from 3 patients who received liver extract with an addition of the vitamins-B (Hepto-B), without effect.

Fig. 43 shows that vitamin-K has no effect.

Fig. 44 shows that aneurin has no effect and fig. 45 shows that neither does lactoflavin lead to any increase in the number of normocytes.

Fig. 38—50: The number of normocytes (in mill. pr.  $\text{mm}^3$ ) in cases of chronic hepatitis. The stippled curves represent the curve of biologic growth and indicate the rise and fall one must expect in the number of normocytes if the treatment is of any significance. The numbers refer to case-numbers in table 46.

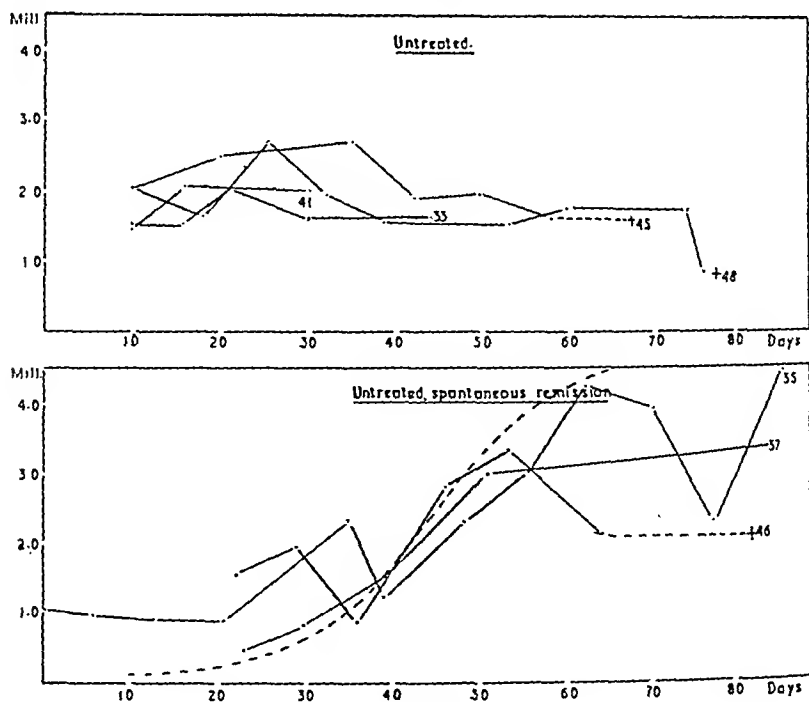


Fig. 38, 39.

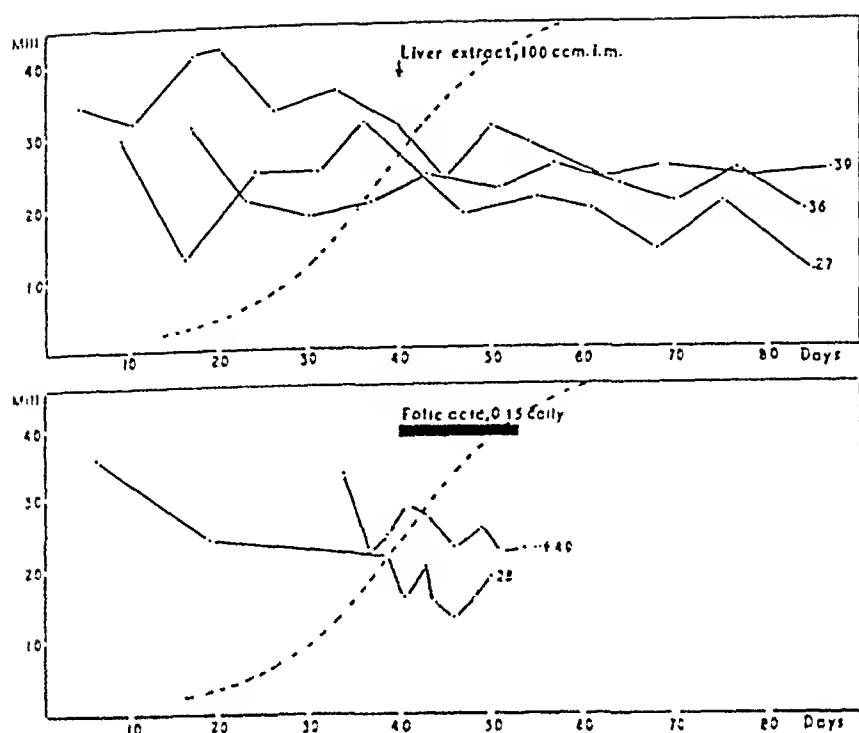


Fig. 40, 41.

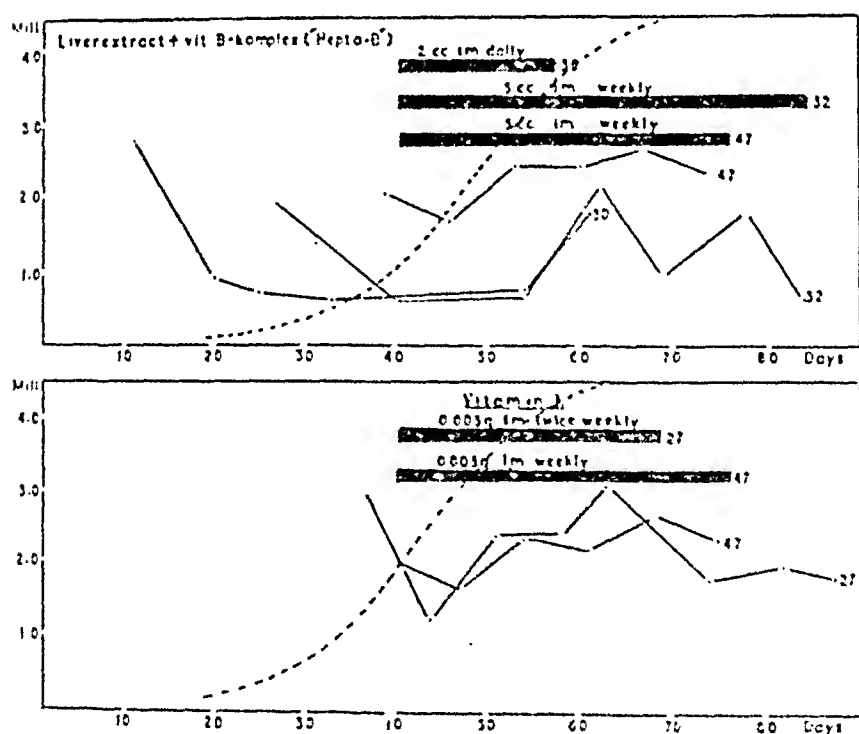


Fig. 42, 43.

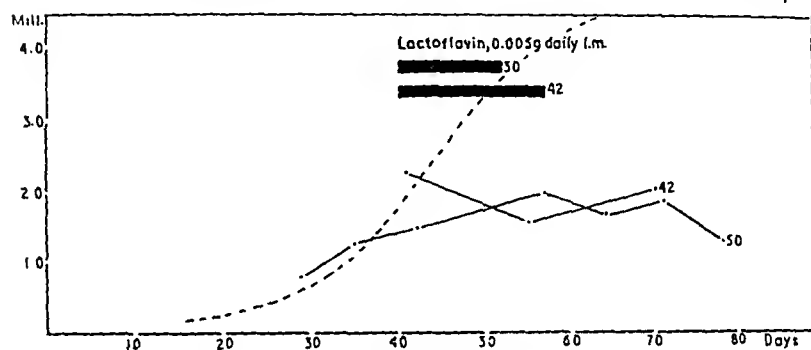
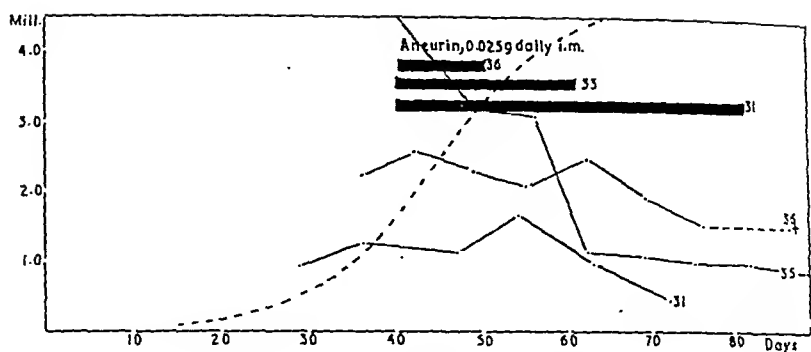


Fig. 44, 45.

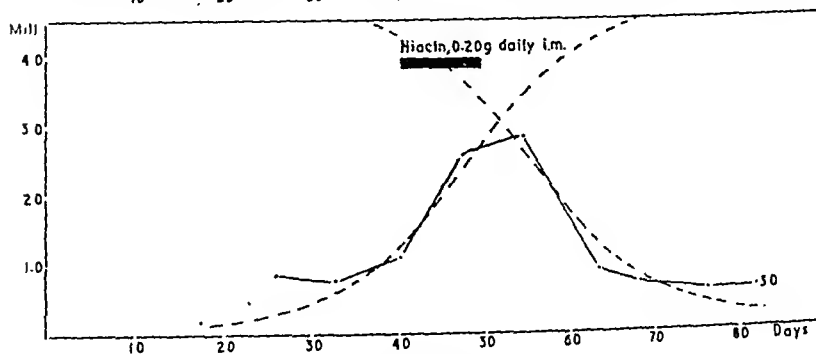
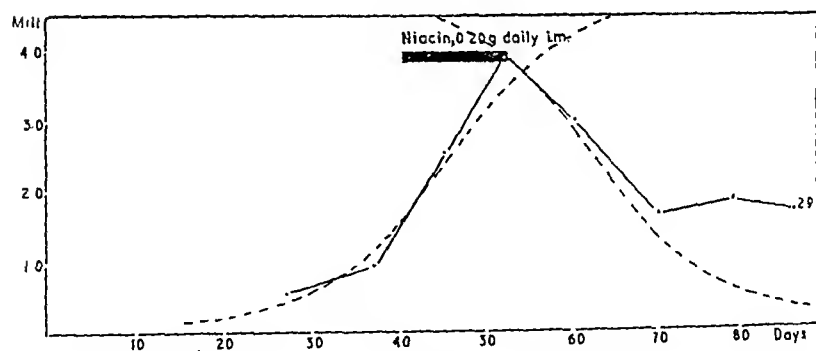


Fig. 46, 47.



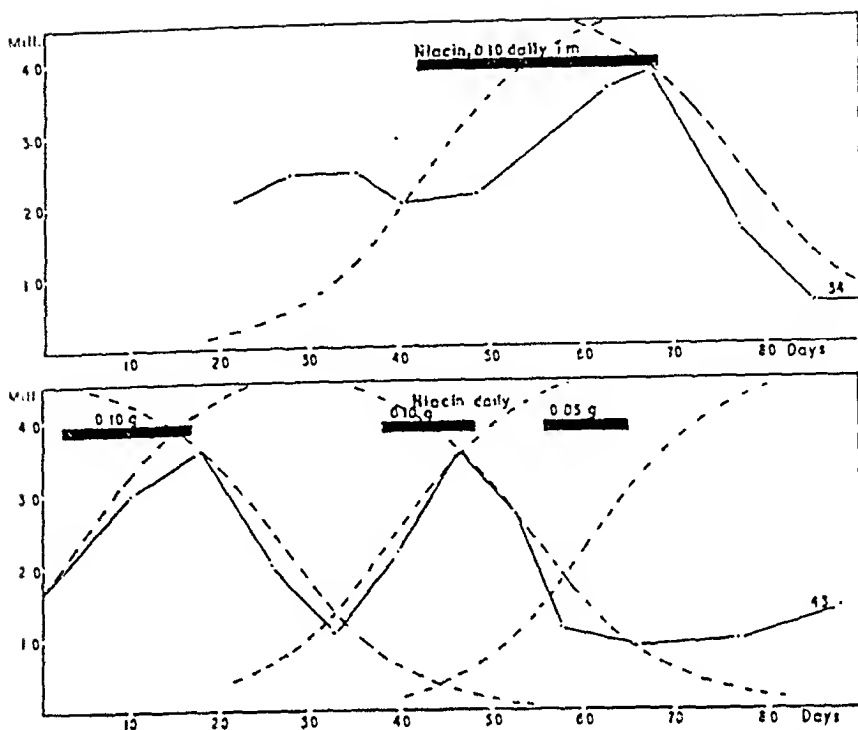


Fig. 48, 49.

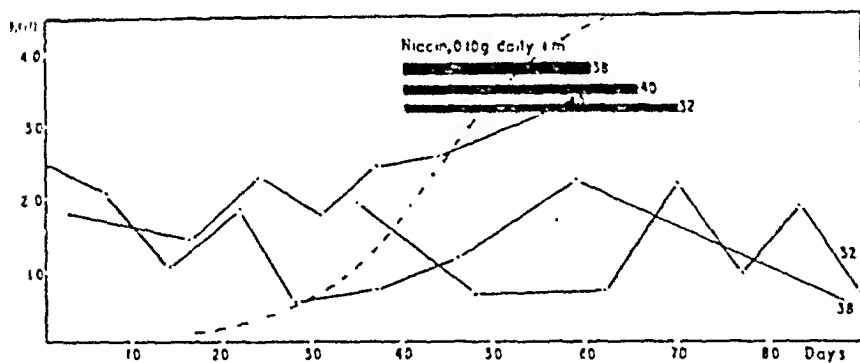


Fig. 50.

Fig. 46 and 47 shows similar curves from patients nos. 29 and 30. These two patients received niacin, 0.20 daily, i. m. The number of normocytes in the blood of these patients increased during the treatment and reached nearly normal values. The increase occurred in close concordance with the biological equation of growth. When the niacin treatment is discontinued the number of normocytes recede to the original values at the expected rate.

Fig. 48 is from patient no. 34. The dose of niacin was in this case only half of the former, 0.10 daily. Even here we find an increase in the number of normocytes, but the increase appears somewhat later than in the two first cases. But when the niacin treatment is discontinued, the normocytes recedes to the original values in concordance with the theoretical curve.

Fig. 49 shows a similar curve from patient no. 43 who received niacin in three periods. The dose was the first two times, 0.10 daily and the increase and the decrease of the normocytes appeared as expected. The third time the dose was only 0.05 gr. daily, and this time the effect was absent.

Finally, fig. 50 shows the curves from 3 patients who also received niacin, but where the effect was absent. In cases nos. 38 and 40 there is an increase in the number of normocytes, but the increase is not in concordance with the theoretical curve and in case no. 40 at least, the rise starts before the treatment is begun. This is probably a case of spontaneous remission. In case no. 32 there was definitely no effect.

These experiments are too few, and give too conflicting results to be regarded as decisive. Niacin does perhaps play some role in the ripening of the macroblast into a normoblast. The reason for the failure in the last three cases may be that the organism needs a sufficiently large dose, and that the niacin is without any effect in insufficient quantities. Perhaps the demonstrated curves are only due to spontaneous remissions and therefore of no consequence.

The question can not be decided without further investigation. One might examine more patients with chronic hepatitis. I have had no opportunity to do this, owing to lack of material. It would be better to investigate the condition of the blood cells in experimentally-caused jaundice. Such an experimentally produced jaundice might also definitely answer the question whether the macrocytosis appears and disappears at the same rate. Even if this is probable, it has not been proved in the present work (see page 156). O. HANSEN and P. HANSEN have shown that one may cause a jaundice resembling acute benign hepatitis by administering lactofenin. I have carried out several such experiments, but have been unable to produce jaundice, perhaps because the lactofenin used was of a poor quality.

Finally one might answer the question by animal experiments, as mentioned by HIGGINS & STASNEY and HEINLE, CASTLE & ROSE. This comes however outside the frame of the present investigation.

### 5) Which diagnostic and prognostic conclusions may be drawn from the presence of macrocytosis?

This, the fifth and last question put forward in the introduction, page 25, may now be answered: The demonstration of macrocytosis in a case of jaundice signifies a disturbed erythropoiesis connected with hepatocellular damage. In jaundice due to occlusion, without simultaneous hepatocellular damage, macrocytosis does not occur. This is borne out by the present investigation which confirms the earlier findings of SCHALM, HAMMARSTEN & CO-WORKERS, and others. HAMMARSTEN and CO-WORKERS have for a number of years studied the relationship between macrocytosis and other signs of liver disease, and have found a close relationship. Recently, LINDGREN (2), in an extensive survey, has shown that the degree of macrocytosis in liver disease is closely related to tests based on the hepatic excretory and secretory actions, as well as on the functions of the liver in the intermediary metabolism. He finds, on the other hand, no significant relation between the red blood cell mean diameter and tests based on the detoxicating ability of the liver, and tests empirically found to be of value in liver diagnostics. The present investigation confirms this, and the presence of macrocytosis may therefore be used both in the diagnosis and the prognosis of liver diseases. If the mean diameter of the red blood cells is definitely increased, whereas the mean corpuscular volume remains normal, it is overwhelmingly probable that hepatocellular damage exist.

But the method has a serious drawback: The range of the normal values for MD is so great that an observed value may be well within normal limits even with manifest macrocytosis. A determination of the mean diameter alone, either by micrometry or by halometry, is therefore only of conditional value. To ascertain if macrocytosis is present, the complete analysis of the frequency curve is necessary. But this involves so much work, that the method can hardly be of any practical value as a method of routine.

errors may however be reduced to such an extent that the measurements become comparable.

*Chapter IV:* The causes of the errors found are discussed. They are partly due to human bias. This leads in some instances to the assistant making the observed frequency curves leptokurtic, in other instances to the curves becoming «dentated» and irregular. Both types of bias occur frequently. Other errors are due to an optical phenomenon, called «the scatter of light by the cell borders». This cause of error is the main reason why the mean diameter of the cells is registered differently with different optical equipment and by different assistants. By photometric studies (fig. 7—10) it is shown that this phenomenon explains the differences in MD found by the statistical analysis in chapter III.

*Chapter V:* Measurement of the blood cell diameter by other methods than projection is discussed. It is shown that direct micrometry of blood films, and measuring in wet preparations, for various reasons give unsatisfactory results: Direct micrometry is so taxing on the eyes and gives so much play for a personal judgment that the method has to be discarded (pg. 77). In wet preparations the cells undergo changes while the measuring is going on, so that the values, especially for the standard deviation, are not comparable (table 26). The cause of the difference in the normal values of earlier authors, given in fig. 1, is discussed. It is shown that the differences may be explained by the sources of errors discovered in chapters III—V.

*Chapter VI* contains a discussion of the frequency curves obtained in pathological cases. It is shown that the abnormal frequency curves reflect real conditions in the pathological blood, and that the curves do not vary more than what might be expected from random sampling errors.

It is shown that these pathological frequency curves probably are heterogeneous, and a method for breaking up such heterogeneous curves is evolved. This method, which is based on a method originally proposed by MOGENSEN, is modified in important ways. The accuracy of the method is determined by random sampling experiments, and the practical use of the method, facilitated by the use of specially prepared auxiliary tables, is illustrated.

*Chapter VII* contains a description of the final measuring technique. A discussion of how many blood cells it is necessary

to measure, based on random sampling of pathological universes, ends with the conclusion that 200 cells is an adequate number.

The normal values for the different characteristics, with the variation to be expected, are given. At the end of the chapter the normal haematological values used are given in tables 33 and 35.

*Part II: Red blood cell diameters in liver disease.*

*Chapter VIII:* The clinical material used in the investigation of the blood cell diameter in liver diseases is reported. It consists of 26 cases of acute hepatitis (duration less than 90 days with full recovery), of 32 cases of chronic hepatitis (duration of more than 90 days), and of 14 cases of cholecystitis or cholelithiasis. (Obstructive jaundice) (table 46). After a brief discussion of the age/sex incidence it is shown that all cases with hepatocellular damage have an increased diameter of the red blood cells, while the mean corpuscular volume remains normal.

*Chapter IX:* The cause of the macrocytosis is by some authors said to be changes in the peripheral blood stream. This hypothesis is discussed, and it is conclusively shown that neither jaundice, nor osmotic disturbances, nor any other peripheral cause previously suggested, can account for the findings in the present material. It is shown (fig. 28, 31 and text pg. 134—136) that the macrocytosis is probably due to the appearance of a distinct new group of larger blood cells in the peripheral blood stream, so that the blood cell population becomes heterogeneous, consisting of a mixture of normal and pathological large cells.

*Chapter X:* Further arguments for the blood cell population being heterogeneous are given. Based on the present material it is calculated that the mean diameter of the hypothetical large cells must average 0.75  $\mu$  higher values than those of the normal cells. Subsequent statistical analysis of the frequency curves, 412 in all, shows this assumption to be correct. On pg. 142—146 further proof is given that the analysis reveals real conditions in the pathological blood samples. It is shown that the pathological large cells are thinner than normal (fig. 32) and more resistant to hypotonic salt solutions (fig. 33).

It is shown that the regeneration of normocytes in acute hepatitis occurs at the same rate as the formation of erythrocytes in pernicious anaemia, and that the regeneration occurs according to

the equation for autocatalytic mono-molecular reactions, the biological growth formula (fig. 36).

*Chapter XI:* The frequency curves obtained in liver diseases are compared with the frequency curves from pernicious anaemia. It is shown that these curves are not identical: While the blood cell population in hepatitis consist of 2 components, the blood cell population in untreated pernicious anaemia consists of at least 3, probably 4 components. When patients with pernicious anaemia are treated with liver extract the blood cell population changes so that it can not be distinguished from that seen in hepatitis. This condition persist for months and years in cases of pernicious anaemia, even when the blood counts become normal.

*Chapter XII:* Contains a résumé of the author's findings, and a discussion of the results found by other authors.

It is concluded that the disturbances seen in hepatitis as well as those seen in pernicious anaemia are due to a disturbed maturation of the red blood cell precursors in the bone marrow. In pernicious anaemia the main disturbance lies at the level of the proerythroblasts, which partly develop into megaloblasts and megalocytes. This disturbance is removed when adequate treatment with liver extract is given.

In liver disease, as well as in treated pernicious anaemia, the disturbance lies at the level of the macroblasts, which develop into macrocytes instead of normally, into normoblasts and normocytes. The cause of this disturbance in the maturation is discussed. Reports made by earlier authors may suggest that lack of a member of the vitamin-B group may be the cause. Some tentative experiments are reported which may indicate that niacin, in one way or another, plays a role. The results are however too conflicting to be decisive.

Finally, the diagnostic and prognostic conclusions which may be drawn from this investigation are briefly stated.

AUXILIARY TABLES FOR THE ANALYSIS  
OF FREQUENCY CURVES

Tables 42—45.

Table of Logarithms.

$$m_0 = + \frac{1}{4} \text{ class-intervall.}$$

(Values of  $m_0$  ending on .187 - .437 - .687 - .937)

$(x+m_0)^2$	Standard-deviation of Normal Curves.									
	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.60
3.28				0.03	0.04	0.07	0.10	0.13		
2.43	0.04	0.07	0.11	0.16	0.22	0.29	0.37	0.49		
1.72	0.28	0.39	0.52	0.64	0.80	0.95	1.10	1.31		
1.12	1.27	1.55	1.86	2.08	2.41	2.69	2.98	3.19		
0.66	4.23	4.63	4.95	5.39	5.74	6.01	6.28	6.50		
0.32	10.02	10.34	10.46	10.66	10.67	10.74	10.71	10.72		
0.10	17.51	17.14	16.85	16.37	15.99	15.58	15.15	14.78		
0.00	22.26	21.27	20.34	19.70	19.01	18.32	17.70	17.17		
0.04	20.52	19.84	19.13	18.51	17.97	17.39	16.94	16.41		
0.19	13.84	13.81	13.71	13.59	13.42	13.31	13.16	12.98		
0.47	6.84	7.17	7.50	7.80	7.94	8.23	8.36	8.51		
0.88	2.46	2.80	3.10	3.50	3.85	4.11	4.41	4.62		
1.41	0.61	0.80	1.02	1.22	1.45	1.65	1.90	2.11		
2.07	0.12	0.18	0.25	0.34	0.42	0.54	0.65	0.79		
2.86	0.02	0.03	0.04	0.07	0.10	0.15	0.20	0.25		
3.76					0.02	0.03	0.05	0.07		
4.80								0.02		

$$m_0 = + \frac{1}{2} \text{ class-intervall.}$$

(Values of  $m_0$  ending on .125 - .375 - .625 - .875)

$(x+m_0)^2$	Standard-deviation of Normal Curves.									
	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.60
4.00						0.02	0.04	0.05		
3.06				0.05	0.07	0.10	0.14	0.18		
2.25	0.08	0.15	0.20	0.24	0.31	0.39	0.46	0.57		
1.56	0.44	0.55	0.70	1.04	1.07	1.26	1.47	1.65		
1.00	1.75	2.07	2.41	2.74	3.03	3.31	3.61	3.88		
0.56	5.41	5.60	6.29	6.52	6.80	7.07	7.20	7.51		
0.25	11.96	11.99	12.07	12.10	12.09	12.07	12.02	11.81		
0.06	19.15	18.84	17.99	17.54	17.07	16.57	16.18	15.66		
0.00	22.42	21.56	20.70	19.85	19.15	18.43	17.72	17.36		
0.06	19.15	18.84	17.99	17.54	17.07	16.57	16.18	15.66		
0.25	11.96	11.99	12.07	12.10	12.09	12.07	12.02	11.81		
0.56	5.41	5.60	6.29	6.52	6.80	7.07	7.20	7.51		
1.00	1.75	2.07	2.41	2.74	3.03	3.31	3.61	3.88		
1.56	0.44	0.55	0.70	1.04	1.07	1.26	1.47	1.65		
2.25	0.08	0.15	0.20	0.24	0.31	0.39	0.46	0.57		
3.06				0.05	0.07	0.10	0.14	0.18		
4.00						0.02	0.04	0.05		



$$\bar{m}_0 = \frac{1}{h} \text{ class-interval.}$$

(Values of  $\bar{m}_0$  ending on .003 - .013 - .03 - .063 - .113)

$(x - \bar{m}_0)^2$	Standard-deviation of Normal Curves.									
4.00	0.47	0.44	0.44	0.49	0.50	0.53	0.54	0.54	0.54	0.54
3.75				0.47	0.47	0.49	0.49	0.49	0.49	0.49
2.05	0.42	0.43	0.44	0.47	0.47	0.49	0.49	0.49	0.49	0.49
2.07	0.42	0.43	0.44	0.47	0.47	0.49	0.49	0.49	0.49	0.49
1.41	0.51	0.50	1.02	1.22	1.45	1.55	1.55	1.55	1.55	1.55
0.83	2.46	2.60	3.10	3.53	3.95	4.31	4.41	4.41	4.41	4.41
0.47	6.84	7.17	7.49	7.82	7.94	8.23	8.36	8.36	8.36	8.36
0.19	13.84	13.81	13.71	13.59	13.48	13.31	13.15	13.09	13.09	13.09
0.04	20.52	19.84	19.13	18.51	17.97	17.37	16.74	16.24	16.41	16.41
0.00	22.26	21.77	20.54	19.70	19.01	18.37	17.70	17.17	17.17	17.17
0.10	17.51	17.14	16.05	15.37	15.00	14.50	13.95	13.47	13.47	13.47
0.32	10.02	10.34	10.49	10.66	10.87	10.74	10.77	10.77	10.77	10.77
0.66	4.23	4.63	4.55	5.39	5.74	6.01	6.28	6.50	6.50	6.50
1.12	1.27	1.55	1.86	2.00	2.41	2.69	2.94	3.10	3.10	3.10
1.72	0.73	0.79	0.52	0.64	0.60	0.66	1.10	1.31	1.31	1.31
2.43	0.04	0.07	0.11	0.15	0.22	0.29	0.37	0.49	0.49	0.49
3.23				0.03	0.04	0.07	0.10	0.15	0.15	0.15

$$\bar{m}_0 = \pm 0 \text{ class-interval.}$$

(Values of  $\bar{m}_0$  ending on .000 - .250 - .500 - .750)

$(x - \bar{m}_0)^2$	Standard-deviation of Normal Curves.									
3.53	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.56	0.56
2.65		0.03	0.05	0.09	0.13	0.16	0.19	0.27	0.27	0.27
1.50	0.25	0.28	0.37	0.40	0.49	0.62	0.74	0.88	0.88	0.88
1.26	0.72	0.87	1.28	1.42	1.65	1.89	2.21	2.42	2.42	2.42
0.77	2.73	3.33	3.20	4.06	4.41	4.73	5.01	5.35	5.35	5.35
0.39	8.01	7.86	8.92	9.09	9.19	9.42	9.40	9.66	9.66	9.66
0.14	15.07	15.17	15.25	15.22	14.93	14.71	14.65	13.96	13.96	13.96
0.02	22.41	21.55	21.10	19.95	19.15	18.43	17.72	17.36	17.36	17.36
0.00										
0.02	22.41	21.55	21.10	19.95	19.15	18.43	17.72	17.36	17.36	17.36
0.14	15.07	15.12	15.25	15.22	14.93	14.71	14.65	13.96	13.96	13.96
0.39	8.01	7.86	8.92	8.98	9.19	9.42	9.40	9.66	9.66	9.66
0.77	2.73	3.33	3.60	4.06	4.41	4.73	5.01	5.35	5.35	5.35
1.26	0.72	0.82	1.28	1.42	1.65	1.89	2.21	2.42	2.42	2.42
1.50	0.16	0.28	0.32	0.38	0.49	0.62	0.74	0.88	0.88	0.88
2.65		0.03	0.05	0.09	0.13	0.16	0.19	0.27	0.27	0.27
3.53						0.04	0.08	0.10	0.10	0.10

Logarithms of numbers to three decimal places.

	0	1	2	3	4	5	6	7	8	9
10	000	004	009	013	017	021	025	029	033	037
1	000	041	079	114	146	176	204	230	255	279
2	301	322	342	362	380	398	415	431	447	462
3	477	491	505	519	531	544	556	568	580	591
4	602	613	623	633	643	653	663	672	681	690
5	699	708	716	724	732	740	748	756	763	771
6	778	785	792	799	806	813	820	826	833	839
7	845	851	857	863	869	875	881	886	892	898
8	903	908	914	919	924	929	935	940	944	949
9	954	959	964	968	973	978	982	987	991	996

# TABLE 46.

## CASE REPORTS

In this table haematological and clinical findings relating to all patients are given, together with the result of the analysis of the frequency curves.

In the left-hand column is given case number, patients initials, sex (M : man, W : woman), birth-date, register-number of hospital case report, and date of admission and discharge from hospital, in order named.

With the exception of case no. 28 and 49, who were treated in The University Clinic, Med. Dept. A, all patients were treated in Norwegian Deacons Hospital, Oslo.

Explanation of abbreviations is given in table of notation, (p. 9—10).

### *Case-numbers to patients excluded from the material: Norwegian Deacons Hospital, Oslo.*

1188/43	1227/44	852/45
19/44	1135/44	1065/45
513/44	1193/44	47/46
548/44	1227/44	216/46
606/44	224/45	300/46
948/44	355/45	318/46
961/44	728/45	678/46
977/44	763/45	807/46
1108/44	852/45	

### *Haematological data used in the discussion in chapter X. Case-numbers to patients with pernicious anaemia. University Clinic, Med. Dept. A.*

4811/27	1894/28	526/32
5919/27	3676/28	1633/32
247/28	2656/29	3666/32
375/28	4498/31	3886/32
602/28	212/32	7960/32

ACUTE HEPATITIS	DATE	Hb.	R. b c	Vol %
No. 1: H. I. M. b. 12/10-09. C. no. 1001/45, 28/8-45—14/9-45. Tired and unwell two weeks before adm. One week before adm. dyspepsia, jaundice. 29/8: R. f.: 0.30/0.40. T.: 0.	29/8	13.8	5.28	40
	30/8	13.8	4.96	40
	7/9	14.1	5.02	43
	13/9	14.6	5.20	43
No. 2: B. S. M. b. 20/11-34. C. no. 26/46, 28/12-45—7/1-46. Father had hepatitis 3 month previously, mother 2 month previously. Patient diabetic since 1942. One week before adm. febrile (38°) 3 days later jaundice. 4/1: A/G: 5.1/2.4. T.: 0.	19/12	14.2	5.10	41
	21/12	13/8	5.12	37
	24/12	13.4	4.64	39
	29/12	13.4	4.98	36
	4/1	12.3	4.52	37
No. 3: A. L. W. b. 23/7-72. C. no. 222/46, 28/1-46—24/2-46. Felt tired and unwell three weeks before adm. Last two weeks dyspepsia. 23/1: Jaundice. Xray: Normal. 29/1: R. f.: 0.26/0.48. R.: 1.7 %. T.: 0.	29/1	13.8	4.54	38
	5/2	13.8	4.82	34
	12/2	13.0	4.66	37
	20/2	13.1	4.64	37
	4/3	13.2	4.67	37
No. 4: A. J. W. b. 16/11-17. C. no. 562/46, 27/6-46—16/7-46. 5 days before adm. febrile, vomited, jaundice. 28/6: R. f.: 0.32/0.50. 30/6: A/G: 4.4/3.1. R.: 0.1 %. T.: 0.	28/6	13.9	5.10	42
	2/7	14.6	5.12	39
	6/7	12.4	4.64	32
	15/7	13.0	4.71	37
No. 5: G. R. M. b. 29/9-89. C. no. 1064/43, 26/8-43—21/9-43. Dyspepsia and diarrhoea in two weeks before adm. Jaundice two days before adm. T.: 0.	27/8	13.4	4.96	39
	30/8	13.8	4.92	40
	2/9	14.5	5.20	42
	6/9	13.8	4.86	42
	9/9	12.7	4.60	36
	13/9	9.9	3.60	28
	20/9	10.0	3.50	28
No. 6: G. T. W. b. 18/12-19. C. no. 1270/44, 4/11-44—1.12-44. 9 days before adm., febrile (39°), 4 days before adm. jaundice. 6/11: R. f.: 0.28/0.44. T.: 0.	6/11	10.6	3.74	31
	16/11	11.6	4.16	34
	22/11	11.2	3.96	33
	27/11	11.4	4.40	34
No. 7: R. M. M. b. 7/10-23. C. no. 213/45, 27/1-45—26/2-45. One week before adm. dyspepsia and fever. Jaundice for three days before adm. During the stay in hospital haematuria and cylindruria for a few days. No other complications. Wells reaction negative. 1/2: R. f.: 0.28/0.44. Tk. — 3 times. T.: 0.	29/1	16.3	6.00	51
	31/1	15.6	5.76	48
	7/2	15.2	5.68	42
	14/2	13.3	5.10	44
	21/2	13.6	4.92	40
	26/2	13.2	5.04	40
No. 8: W. T. W. b. 22/3-14. C. no. 242/46, 29/1-46—1/3-46. Parturition 4 month before adm. Fever and dyspepsia a week before adm. Jaundice 5 days before adm. 20/2: A/G: 4.3/3.0. Tk. — T.: 0.	30/1	14.4	5.24	42
	7/2	13.0	4.74	41
	15/2	12.7	4.72	36
	21/2	13.0	4.84	38
	1/3	13.0	5.00	36
No. 9: I. C. W. b. 15/4-02. C. no. 956/46, 24/6-46—14/8-46. Bronchitis 4 month before adm. Then well till 8 days before adm. when febrile. 2 days before adm. Jaundice. 28/6: R. f.: 0.22/0.40. T.: 0.	25/6	12.2	4.58	34
	28/6	12.4	4.36	30
	3/7	12.3	4.44	31
	12/7	12.6	4.40	30
	17/7	12.7	4.32	34
	13/8	12.5	4.62	34
No. 10: B. L.-B. W. b. 24/10-74. C. no. 509/45, 23/4-45—11/5-45. Two month before adm. dyspepsia, jaundiced. Lost in weight. At adm. was the liver distinctly felt. Tk. — on repeated occ. 24/4: R. f.: 0.22/0.44. T.: 0.	23/4	13.0	4.60	34
	26/4	12.7	4.68	34
	28/4	12.5	4.60	34
	2/5	12.5	4.58	34
	7/5	12.4	4.62	37
	11/5	11.6	4.14	38

Corp vol.	MCH	MCC	MD	s	m <sub>l</sub>	N <sub>l</sub> %	m <sub>r</sub>	N <sub>r</sub> %	s <sub>l</sub>	s <sub>r</sub>	$\chi^2$	I. I.	S. R.
77	26.1	34.5	8.30	0.64	7.88	50	8.77	50	0.48	-	2.93	-	-
81	27.8	34.5	8.41	0.61	8.00	52	8.84	48	0.43	-	0.99	46	16
86	28.1	37.8	8.23	0.53	8.00	68	8.83	32	0.47	-	6.77	-	-
83	28.1	34.0	7.96	0.48	7.81	80	8.53	20	0.43	-	1.35	12	36
80	27.9	34.7	8.48	0.80	8.00	49	8.74	51	0.44	-	7.72	35	22
73	27.0	37.4	8.43	0.50	8.13	59	8.84	41	0.41	-	8.26	-	-
84	29.0	34.4	8.27	0.57	8.13	74	8.75	26	0.49	-	1.28	16	-
73	26.9	37.2	8.30	0.54	8.13	71	8.85	29	0.44	-	0.40	-	-
82	27.2	33.2	8.12	0.48	8.12	100	-	-	-	-	0.89	7	14
84	30.2	36.2	8.40	0.63	7.77	25	8.56	75	-	0.48	11.75	26	81
70	28.6	40.5	8.32	0.56	8.00	44	8.63	56	-	0.47	3.45	10	95
73	27.8	35.1	8.00	0.53	7.88	73	8.41	27	0.45	-	4.08	-	-
80	28.2	35.4	8.00	0.53	7.88	80	8.44	20	0.48	-	3.78	8	76
79	28.2	35.7	7.94	0.47	7.94	100	-	-	-	-	4.27	-	-
83	27.2	33.0	8.51	0.50	8.13	14	8.56	86	-	0.47	1.98	30	54
76	28.5	37.2	8.65	0.57	8.10	30	8.88	70	-	0.45	0.49	38	47
69	26.7	38.7	8.43	0.58	8.00	48	8.80	52	0.46	-	0.57	17	36
78	27.6	35.2	8.19	0.57	8.00	76	8.85	24	0.46	-	1.37	7	45
79	27.0	34.6	8.48	0.48	-	-	8.48	100	-	0.48	0.60	20	22
81	28.1	34.5	8.37	0.58	7.73	20	8.56	80	-	0.46	4.55	-	-
81	27.9	34.5	8.64	0.52	7.90	20	8.81	80	-	0.48	2.13	82	22
87	28.4	32.8	8.51	0.55	7.81	23	8.63	77	-	0.48	1.19	-	-
78	27.6	35.2	8.35	0.59	7.77	39	8.63	61	-	0.49	7.79	30	21
78	27.5	35.4	7.97	0.51	7.88	87	8.69	13	0.44	-	2.04	-	-
80	28.6	35.6	7.80	0.44	7.80	100	-	-	-	-	1.15	18	30
83	28.3	34.2	8.40	0.60	7.49	10	8.44	90	-	0.49	2.10	115	12
82	27.9	34.1	7.99	0.52	7.77	37	8.25	63	-	0.44	5.50	30	48
83	28.3	34.2	7.56	0.62	7.38	80	8.33	20	0.50	-	1.34	22	50
78	25.9	33.6	7.88	0.52	7.75	85	8.54	15	0.47	-	1.95	12	34
85	27.2	32.0	8.51	0.60	7.81	22	8.75	78	-	0.46	2.57	-	2
83	27.0	32.5	8.64	0.53	8.12	25	8.81	75	-	0.47	1.49	66	-
84	26.8	31.5	8.48	0.53	8.12	60	8.85	30	0.46	-	1.84	64	20
86	26.1	30.2	8.07	0.51	7.82	69	8.58	31	0.44	-	0.32	32	-
81	27.6	34.0	7.96	0.54	7.88	91	8.77	9	0.48	-	1.06	16	-
79	26.2	33.0	7.95	0.46	7.95	100	-	-	-	-	1.22	-	-
80	27.5	34.3	8.42	0.53	7.73	18	8.56	82	-	0.44	2.57	32	16
86	27.4	31.7	8.35	0.56	7.88	35	8.63	65	-	0.45	3.02	50	-
77	26.9	35.2	8.09	0.55	7.88	75	8.63	25	0.48	-	0.32	26	-
79	26.9	34.2	8.00	0.46	8.00	95	Graphic				1.13	15	23
72	26.0	36.2	7.81	0.49	7.81	95	Graphic				4.27	10	9
73	26.6	35.9	8.80	0.61	8.26	30	9.06	70	-	0.46	0.91	8	13
69	28.4	41.4	8.81	0.59	8.33	38	9.13	64	-	0.46	4.23	32	18
70	27.8	39.7	8.74	0.58	8.24	34	9.00	66	-	0.43	4.54	-	-
68	28.8	42.0	8.45	0.53	8.25	67	8.96	33	0.45	-	5.04	14	63
79	29.4	37.4	8.36	0.50	8.19	73	8.75	27	0.47	-	4.97	10	65
74	27.1	36.8	8.32	0.44	8.32	100	-	-	-	-	1.25	5	60
74	28.3	38.3	8.77	0.66	7.93	20	9.00	80	-	0.50	6.29	43	17
73	27.2	37.3	8.44	0.58	7.99	29	8.63	71	-	0.45	3.14	22	31
74	27.2	36.8	8.35	0.56	7.74	25	8.56	75	-	0.46	0.44	22	26
74	27.3	36.8	8.45	0.59	8.00	31	8.63	69	-	0.47	2.20	26	39
80	26.8	33.5	8.22	0.57	7.94	58	8.63	42	0.48	-	1.96	-	-
92	28.0	30.5	8.04	0.58	7.88	78	8.71	22	0.44	-	0.57	4	48

ACUTE HEPATITIS		DATE	Hb.	R. b. c.	Vol. %
No. 11: M. B. W. b. 28/10-85. C. no. 241/46, 4/2-46—1/3-46. Jaundiced for some days 2½ month before adm. Again jaundice 3 weeks before adm. 5/2: R. f.: 0.30/0.44, A/G 4.2/3.2, A/G 20/2: 3.7/3.6. Tk. — twice. T.: 0.		5/2 12/2 20/2 26/2	14.4 14.1 13.3 13.2	5.21 5.00 4.58 4.68	41 39 36 36
No. 12: K. F. W. b. 5/1-01. C. no. 899/46, 29/6-46—28/7-46. Jaundice 3—4 weeks before adm. Slight fever. 1/7: R. f.: 0.20/0.42, A/G 3.5/2.4. Tk. —. T.: 0.		1/7 5/7 15/7 24/7	10.8 9.7 9.4 —	4.00 3.76 3.76 —	31 28 28 —
No. 13: R. J. W. b. 27/10-27. C. no. 127/44, 20/12-43—0/2-44. Felt tired and unwell for 3 weeks before adm. Jaundiced about 2 weeks before adm. At adm. jaundiced. She had also diabetes treated with insulin. Terapi 0.		21/12 27/12 30/12 5/1 12/1 19/1 25/1 8/2	13.0 12.3 11.6 11.6 11.8 12.0 12.7 —	4.90 4.50 4.30 4.40 4.40 4.40 4.34 —	37 39 35 35 35 35 34 —
No. 14: K. S. M. b. 10/9-99. C. no. 250/45, 7/1-45—5/3-45. <i>Diagnosis: Weil's disease.</i> Tired, febrile, and jaundiced two weeks before adm. In hospital jaundice, albuminuria, cylinduria, aggl. test. Well + 1/800. At the time of discharge normal findings in blood and urine. Tk. —. T.: 0.		8/1 15/1 23/1 30/1 6/2 12/2 19/2 26/2	13.5 12.2 10.2 10.8 10.8 11.2 11.2 12.8	4.28 4.40 4.08 3.80 3.76 4.20 4.02 4.70	35 37 31 32 31 30 32 38
No. 15: M. J. W. b. 4/11-70. C. no. 74/45, 8/12-11—20/1-45. <i>Diagnosis: Weil's disease.</i> Felt unwell 10 days before adm. Jaundice 6 days b. a. Findings: Jaundice, albuminuria, cylinduria, aggl. test Well + 1/800. Blood urea 114 mg %—85 mg % Tk. —. Normal findings in blood and urine at the time of discharge. T.: 0.		9/12 18/12 23/12 28/12 3/1 10/1 18/1	14.6 9.9 9.7 9.4 9.3 9.4 9.4	5.54 3.60 3.48 3.62 3.40 3.46 3.22	47 31 29 29 26 30 29
No. 16: N. D. M. b. 20/10-25 C. no. 188/43, 4/2-43—20/2-43. Fever and jaundice two days before adm.		5/2	16.6	6.00	50
No. 17: G. O. W. b. 24/6-16. C. no. 1304/43, 2/11-43—7/11-43. Fever and malaise two weeks, jaundice 2 days before adm.		2/11	15.0	5.44	45
No. 18: N. D. W. b. 17/1-01. C. no. 1300/43, 20/11-43—20/11-43. Three children jaundice recently. Fever and jaundice 6 days b. a.		20/11	13.3	4.92	45
No. 19: B. F. M. b. 2/7-75. C. no. 92/44, 11/1-44—20/1-44. Jaundice and dyspepsia for 3 weeks. R.: 1.4 %.		12/1	10.5	3.92	32
No. 20: S. N. W. b. 18/12-22. C. no. 323/44, 14/3-44—21/3-44. Dyspepsia and jaundice 4 days before adm.		14/3	13.5	4.94	40
No. 21: I. J. J. W. b. 10/10-11. C. no. 464/44, 28/3-44—24/4-44. Malaise for 2 weeks. Jaundice 5 days before adm.		28/3	15.2	5.48	47
No. 22: K. J. M. b. 7/5-26. C. no. 786/44, 4/7-44—15/7-44. Mother and maid jaundice 3 weeks previously. Jaundice 1 week.		5/7	11.7	4.50	38
No. 23: K. H. H. M. b. 30/9-81. C. no. 851/44, 17/7-44—5/8-44. Diabetes 1931. Tired last month. Jaundice 2 days before adm.		17/7	13.8	4.86	39
No. 24: I. O. W. b. 28/8-23. C. no. 1443/45, 10/12-45—21/12-45. Malaise one month b. a. Last two days: Jaundice.		20/12	13.5	4.88	39
No. 25: L. Y. W. b. 28/12-86. C. no. 513/46, 6/7-45—1/8-45. Jaundice two weeks before adm. R. f.: 0.26/0.40.		7/7	13.3	4.84	40
No. 26: A. H. W. b. 28/5-10. C. no. 544/46, 23/4-46—8/5-46. Jaundiced three weeks, R. f.: 0.24/0.40, A/G. 2.5/3.3.		24/5	12.2	4.18	32

Corp vol	MCH	MCC	MD	s	m <sub>1</sub>	N <sub>1</sub> %	m <sub>r</sub>	N <sub>r</sub> %	s <sub>1</sub>	s <sub>r</sub>	$\chi^2$	I. I.	S. R.
79	27.6	35.1	8.76	0.64	8.03	25	9.00	75	-	0.49	3.67	30	10
78	28.2	36.2	8.30	0.61	7.88	55	8.78	45	0.44	-	1.81	16	10
79	29.1	37.0	7.95	0.59	7.75	80	8.69	20	0.46	-	0.80	11	25
77	29.5	38.4	8.04	0.57	7.94	87	8.57	13	0.49	-	3.14	9	22
77	27.0	34.8	8.71	0.68	8.18	44	9.13	55	-	0.44	2.75	23	16
74	25.8	34.5	8.41	0.63	8.13	68	8.93	32	0.46	-	2.39	-	-
75	25.2	33.5	8.06	0.51	8.06	100	-	-	-	-	9.10	16	10
-	-	-	8.08	0.54	8.08	100	-	-	-	-	2.29	16	20
76	26.5	35.0	8.36	0.55	7.58	17	8.50	83	-	0.45	0.89	61	46
87	27.4	31.5	8.06	0.57	7.75	55	8.46	45	0.46	-	6.63	-	-
82	27.0	33.2	7.77	0.63	7.50	70	8.46	30	0.48	-	1.54	-	-
80	26.4	33.2	7.89	0.56	7.75	71	8.37	29	0.45	-	3.17	25	84
80	27.0	33.8	7.53	0.52	7.53	95	Graphic	-	-	-	2.64	21	38
80	27.3	34.2	7.50	0.51	7.50	100	-	-	-	-	4.21	-	-
79	29.2	37.2	7.72	0.50	7.72	100	-	-	-	-	3.57	18	12
-	-	-	7.35	0.51	7.35	100	-	-	-	-	4.52	16	10
82	31.5	38.5	8.64	0.54	7.85	20	8.67	80	Graphic	-	4.35	55	104
84	27.6	33.0	8.22	0.60	7.72	31	8.44	69	-	0.50	5.60	52	112
76	25.0	33.0	8.04	0.56	7.81	65	8.45	35	0.50	-	3.55	-	-
84	28.4	33.8	7.75	0.52	7.69	90	8.18	10	0.48	-	1.01	26	106
82	28.7	34.8	7.48	0.53	7.48	100	-	-	-	-	2.95	15	53
72	26.7	37.3	7.71	0.55	7.56	80	8.25	20	0.49	-	1.37	10	75
80	27.9	35.0	7.69	0.47	7.69	100	-	-	-	-	1.19	-	40
81	27.2	33.7	7.62	0.46	7.62	100	-	-	-	-	4.06	-	-
85	26.4	31.1	8.88	0.60	8.27	27	9.13	73	-	0.46	1.13	65	46
86	27.5	31.9	8.75	0.56	8.38	45	9.06	55	-	0.45	1.75	31	112
84	27.0	33.4	8.41	0.51	8.25	79	9.00	21	0.46	-	5.11	-	-
80	26.0	32.4	8.30	0.52	8.19	80	8.76	20	0.49	-	1.71	22	137
76	27.3	35.7	8.38	0.52	8.25	83	8.99	17	0.48	-	0.59	-	-
86	27.2	31.3	8.25	0.51	8.19	90	8.73	10	0.47	-	1.00	-	-
84	29.2	32.4	8.18	0.61	8.00	75	8.80	25	0.46	-	3.09	9	112
84	27.7	33.2	8.27	0.53	8.10	75	8.85	25	0.44	-	3.09	35	3
83	27.6	33.4	8.64	0.59	8.38	65	9.13	35	0.49	-	4.41	76	6
91	27.1	33.4	8.28	0.55	7.78	35	8.53	65	-	0.48	9.46	64	31
82	26.8	32.8	8.46	0.52	8.30	65	9.05	35	0.50	-	5.08	24	8
81	27.4	33.8	8.43	0.53	8.25	75	9.03	25	0.42	-	0.15	18	20
86	27.8	32.3	7.93	0.72	7.42	53	8.50	47	-	0.45	0.53	65	3
85	26.0	30.8	8.17	0.51	8.00	69	8.67	31	0.44	-	1.32	20	4
80	28.3	35.4	8.87	0.64	8.25	40	9.18	60	-	0.50	0.21	42	12
80	27.7	34.6	8.78	0.68	8.05	30	9.05	70	-	0.44	6.65	11	9
83	27.6	33.2	7.97	0.58	7.75	75	8.53	25	0.48	-	3.60	26	14
77	29.2	38.2	8.72	0.64	8.13	40	9.13	60	-	0.42	0.37	27	21

CHRONIC HEPATITIS		DATE	Hb.	R. b. c.	Vol %
No. 27: A.-M. S. W. b. 10/11-93. C. no. 1113/45, 9/7-45—12/10-45. Previously treated for spondyloarthritis and neurasthenia. Readmitted 9/7-45. Treatment (against spondyloarthritis) started with salazopyrin (Combination of sulfapyridine and salicyl). 7 days later: Jaundice (Toxic?) Tk. —. T.: 22/8: Liver extract 100 ccm. 26/7—23/8: Vitamin K 0.01 i. m. twice weekly.		23/7	12.4	4.44	39
		30/7	13.3	4.52	40
		6/8	13.1	4.56	39
		13/8	12.2	4.52	35
		18/8	12.4	4.82	36
		29/8	11.6	4.14	32
		6/9	11.2	4.08	33
		12/9	11.3	4.12	34
		19/9	13.8	4.50	35
		26/9	13.2	4.62	41
		6/10	12.2	4.08	36
		5/11	11.3	4.06	33
No. 28: A. B. M. b. 29/11-17. C. no. R. H. 8425/47, 4/2-47—21/3-47. No previous illness. Fever (influenza) with jaundice 4 weeks before adm. T.: 11/3—21/3: Folic acid 0.05 × 3 p. o.		5/2	14.6	5.38	41
		18/2	14.6	5.30	40
		10/3	14.5	5.45	41
		12/3	14.3	5.10	40
		14/3	14.5	5.02	39
		15/3	14.5	4.98	37
		17/3	14.5	5.17	39
		19/3	14.5	5.09	37
		21/3	14.6	5.24	39
No. 29: P. T. M. b. 12/12—75. C. no. 349/46, 19/1-46—25/3-46. Jaundice for 6 month before adm., more pronounced the last 3 weeks. 21/1: R. f.: 0.26/0.46. R.: 0.3 %. T. — 4 times. 8/2: A/G.: 4/2.3, 22/3: 3.5/2.1. T.: 3/2—15/2: Niacin 0.20 i. m. daily.		21/1	9.9	4.20	40.
		24/1	11.7	4.14	-
		31/1	12.3	4.40	39
		8/2	10.1	3.50	34
		14/2	-	-	-
		15/2	11.6	4.14	31
		23/2	11.1	4.02	29
		5/3	10.8	4.16	31
		14/3	11.1	4.18	31
		22/3	10.8	4.36	28
No. 30: G. H. W. b. 10/3-99. C. no. 803/46, 12/4-46—1/7-46. 14 year old: Haematemesis. 17 year old: Tuberculous peritonitis. Last 4 month dyspepsia with jaundice, more pronounced last 6 weeks. At admission: Jaundice, ascites, pleuritis. 28/4: Rapid developing coma hepaticum, treated with blood transfusions and niacin. Recovered. No other complications. 13/4: R. f.: 0.28/0.42, 15/6: R. f.: 0.26/0.42, 11/9: R. f.: 0.28/0.42. 13/4: R.: 1.2 %, 13/4: A/G.: 2.7/3.1, 25/5: A/G.: 2.8/2.5. Tk. : +++ at adm., later +. T.: 28/4-8/5: Niacin 0.20 i. m. daily. 10/6—27/6: Tonipan (Liverextract + aneurin) 2 ccm. daily.		13/4	11.2	4.36	31
		20/4	11.6	4.22	31
		27/4	12.2	4.32	33
		4/5	14.5	5.06	36
		11/5	11.6	4.14	33
		20/5	11.8	4.10	33
		25/5	11.8	4.12	32
		3/6	12.2	4.30	33
		8/6	12.6	4.52	31
		15/6	11.8	4.08	30
		24/6	11.8	4.24	30
		1/7	13.6	4.66	35
No. 31: M. S. W. b. 21/12-92. C. no. 426/45, 3/3-45—24/4-45. Jaundice for 3 month before adm. 5/3: R. f.: 0.36/0.44. Tk.: ++++. T.: 16/3-27/4: Aneurin 0.025 g. daily i. m.		5/3	12.7	4.60	39
		12/3	12.4	4.40	38
		23/3	11.1	4.12	33
		31/3	12.2	4.38	36
		9/4	12.0	4.24	34
		18/4	11.9	4.20	32
No. 32: M. C. W. b. 1/4-94. C. no. 240/46, 14/12-45—1/3-49. 1942: Ulcus ventriculi. 1943: Jaundice + fever for two month, diagnosed as cholelithiasis. Again jaundiced for one month before adm. Tk. ++ 20/12: A/G.: 4/3.9, 6/2: A/G.: 3.7/3.7, 27/2: A/G.: 4 3.2. T.: 20/12—19/1: Niacin 0.10 i. m. daily. 28/12—1/3: Hepto-B (Liver extract with vit. B) 5 ccm. weekly.		15/12	13.6	4.78	37
		28/12	12.7	4.68	35
		11/1	11.8	4.42	31
		19/1	12.0	4.40	31
		26/1	11.1	3.95	32
		4/2	12.7	4.64	35
		11/2	11.8	4.32	34
		20/2	13.0	4.60	36
		27/2	13.3	4.50	36



Corp. vol.	MCH	MCC	MD	s	m <sub>1</sub>	N <sub>1</sub> %	m <sub>r</sub>	N <sub>r</sub> %	s <sub>1</sub>	s <sub>r</sub>	$\chi^2$	I. I.	S. R.
88	28.0	31.8	7.45	0.54	7.25	66	7.84	34	0.47	-	3.64	38	-
89	29.4	33.2	7.90	0.63	7.34	28	8.00	62	-	0.42	28.75	110	-
88	28.7	33.6	7.81	0.68	7.31	54	8.13	46	0.46	-	3.92	94	-
78	27.0	34.8	7.62	0.61	7.25	54	8.05	46	0.49	-	0.90	70	30
75	25.7	34.5	7.57	0.69	7.31	65	8.18	35	0.50	-	2.88	78	-
77	28.1	36.2	7.80	0.61	7.33	44	8.19	56	-	0.48	3.82	60	30
81	27.5	34.0	7.83	0.69	7.35	50	8.13	50	0.48	-	11.02	52	-
83	27.4	33.2	7.76	0.69	7.31	46	8.19	54	-	0.48	15.29	28	20
78	30.7	39.4	7.82	0.62	7.18	30	8.13	70	-	0.44	2.13	20	7
89	28.6	32.2	7.64	0.57	7.31	59	8.06	41	0.47	-	4.67	17	-
88	29.9	34.0	7.82	0.60	7.29	25	8.06	75	-	0.48	2.88	-	-
81	27.8	34.3	7.79	0.55	7.29	29	8.00	71	-	0.48	5.84	-	-
76	27.2	35.6	7.46	0.59	7.13	65	7.93	35	0.46	-	6.79	60	-
75	27.6	36.5	7.63	0.68	7.10	45	8.05	55	-	0.50	2.14	15	-
75	26.6	35.4	7.42	0.66	7.00	40	7.75	60	-	0.48	4.25	16	-
78	28.0	35.7	7.63	0.65	7.13	30	7.93	70	-	0.48	4.34	-	-
78	28.9	37.2	7.64	0.66	7.20	40	7.92	60	-	0.50	1.20	-	-
74	29.2	39.2	7.53	0.57	6.93	30	7.75	70	-	0.46	8.19	-	-
75	28.1	37.2	7.50	0.60	6.95	25	7.68	75	-	0.44	11.14	12	-
73	28.5	39.2	7.58	0.60	7.05	30	7.83	70	-	0.49	3.16	-	-
75	27.7	37.2	7.35	0.60	6.90	35	7.68	65	-	0.46	2.28	-	-
96	23.6	24.8	8.41	0.61	7.63	13	8.50	87	-	0.51	0.95	11	39
-	28.5	-	8.40	0.58	7.71	17	8.50	83	-	0.48	2.11	16	55
89	28.0	31.6	8.24	0.51	7.71	22	8.38	78	-	0.42	2.97	20	72
97	28.8	29.7	7.91	0.52	7.75	72	8.62	26	0.43	-	1.21	-	-
-	-	-	7.89	0.42	7.89	100	-	-	-	-	2.44	-	-
75	28.1	37.4	7.83	0.51	7.81	95	8.84	5	0.42	-	0.61	14	75
73	27.7	38.3	7.89	0.67	7.63	75	8.73	25	0.49	-	1.82	12	55
75	28.3	38.0	8.37	0.67	7.76	40	8.81	60	-	0.46	10.51	13	55
74	26.6	35.8	8.27	0.65	7.73	44	8.69	55	-	0.47	3.60	-	-
69	24.7	38.7	8.33	0.57	7.80	39	8.69	61	-	0.44	1.58	6	95
71	25.7	36.1	8.88	0.60	8.20	20	9.06	80	-	0.43	1.86	27	24
73	27.5	37.4	8.85	0.52	8.15	18	9.00	82	-	0.42	6.31	-	19
76	28.2	36.9	8.88	0.57	8.25	25	9.13	75	-	0.43	2.86	45	16
71	28.6	40.3	8.49	0.62	8.15	50	8.87	50	0.49	-	3.64	33	12
80	29.0	35.2	8.40	0.51	8.13	67	8.92	33	0.40	-	5.76	-	12
80	28.2	35.7	8.78	0.56	8.16	22	8.93	78	-	0.45	3.81	14	13
78	28.6	37.0	8.77	0.47	8.05	17	8.88	83	-	0.41	4.74	-	15
77	28.4	37.0	8.88	0.54	7.93	14	9.00	86	-	0.48	1.93	8	33
69	27.9	40.6	8.56	0.51	7.91	14	8.69	86	-	0.46	0.90	-	37
74	28.8	39.3	8.81	0.60	7.95	17	8.94	83	-	0.42	3.17	-	34
71	27.8	39.3	8.67	0.55	8.04	17	8.81	86	-	0.48	1.31	-	45
75	29.2	38.9	8.79	0.55	8.32	39	9.13	61	-	0.44	11.99	-	-
85	27.6	32.6	8.51	0.52	8.02	20	8.63	80	-	0.49	11.17	32	26
86	28.2	32.6	8.53	0.57	7.80	28	8.75	75	-	0.48	1.54	35	31
80	27.0	33.6	8.32	0.52	7.86	27	8.50	73	-	0.47	1.64	21	68
82	27.8	33.9	8.46	0.56	8.01	37	8.75	65	-	0.50	9.15	17	71
80	28.3	35.4	8.36	0.53	7.81	22	8.50	75	-	0.46	3.55	10	36
76	28.4	37.2	8.52	0.54	7.78	10	8.63	90	-	0.49	4.64	10	88
73	28.4	36.8	8.26	0.55	7.86	40	8.50	60	-	0.48	9.99	22	23
72	26.0	36.3	8.34	0.52	7.82	14	8.44	86	-	0.47	1.10	28	38
70	26.8	38.0	8.33	0.57	7.53	16	8.50	84	-	0.43	4.65	13	39
70	27.3	38.5	7.89	0.60	7.51	50	8.25	50	-	0.48	2.33	13	39
81	28.0	34.7	8.15	0.63	7.40	23	8.38	77	-	0.43	0.75	11	43
76	27.4	36.2	8.18	0.63	7.70	40	8.50	60	-	0.49	2.23	10	44
79	27.4	32.8	8.05	0.52	7.63	14	8.11	86	-	0.48	1.26	8	30
78	28.2	36.1	7.82	0.58	7.27	25	8.00	75	-	0.47	3.47	7	25
80	29.6	37.0	8.06	0.66	7.38	30	8.31	70	-	0.46	4.75	7	28

Corp vol	MCH	MCC	MD	s	m <sub>1</sub>	N <sub>1</sub> %	m <sub>r</sub>	N <sub>r</sub> %	s <sub>1</sub>	s <sub>r</sub>	$\chi^2$	I. I.	S. R.
86	30.4	34.9	8.25	0.61	7.81	47	8.57	53	0.47	-	6.84	44	55
87	30.5	35.2	8.09	0.71	7.63	46	8.49	54	0.46	-	3.81	34	-
85	28.7	33.6	7.95	0.65	7.50	51	8.42	49	0.50	-	3.97	28	-
84	29.9	35.8	7.98	0.62	7.52	45	8.38	55	-	0.48	1.53	18	30
-	-	-	7.97	0.52	7.64	47	8.25	53	-	0.45	7.34	-	-
84	28.4	33.8	8.20	0.61	7.79	44	8.50	56	-	0.51	6.80	14	33
88	28.9	33.0	8.64	0.50	8.02	15	8.75	85	-	0.47	3.43	69	30
88	28.6	32.5	8.69	0.55	8.18	30	8.94	70	-	0.48	13.63	-	-
83	26.5	32.0	8.65	0.72	8.19	54	9.25	46	0.49	-	4.18	62	60
84	27.0	32.1	8.52	0.58	8.13	51	8.92	49	0.41	-	2.13	75	56
84	28.3	33.7	8.24	0.70	7.88	55	8.73	45	0.47	-	5.16	-	-
82	27.8	34.1	8.36	0.57	8.00	55	8.75	45	-	0.46	3.24	102	-
83	28.1	34.0	8.58	0.63	8.16	50	9.00	50	-	0.45	1.73	78	73
77	27.7	35.8	8.22	0.63	7.84	50	8.63	50	-	0.48	2.04	35	50
84	26.8	31.7	8.04	0.58	7.88	75	8.53	25	0.52	-	6.12	25	-
82	28.6	35.1	8.08	0.57	7.94	85	8.85	15	0.47	-	2.65	-	77
79	25.8	32.9	8.03	0.53	7.94	90	8.67	10	0.48	-	0.95	16	-
76	28.3	37.4	8.27	0.68	7.75	42	8.63	58	-	0.47	1.30	13	104
77	30.4	39.4	8.47	0.56	7.88	16	8.56	84	-	0.45	5.30	-	-
74	26.0	35.2	8.11	0.52	8.06	84	8.68	16	0.45	-	5.79	4	67
80	26.2	32.0	7.90	0.49	7.90	100	-	-	-	-	2.70	5	55
76	27.7	36.6	7.67	0.49	7.67	100	-	-	-	-	10.05	30	43
72	28.6	39.7	7.80	0.62	7.63	74	8.60	16	0.44	-	8.08	62	-
75	28.4	38.2	7.89	0.54	7.63	71	8.40	29	0.45	-	2.82	42	43
88	29.4	33.7	8.26	0.60	7.69	32	8.50	68	-	0.46	0.55	31	35
83	27.2	32.6	8.03	0.62	7.48	25	8.25	75	-	0.48	5.02	16	37
77	24.4	31.8	8.28	0.51	7.47	21	8.44	79	-	0.45	2.74	16	28
78	24.5	31.7	8.19	0.57	7.47	20	8.38	80	-	0.47	1.15	12	41
87	28.5	32.8	8.57	0.55	7.83	20	8.75	80	-	0.48	1.41	-	-
85	28.4	33.5	8.42	0.60	8.06	54	8.88	46	0.50	-	3.22	18	54
76	28.5	34.2	8.66	0.61	8.10	30	8.88	70	-	0.46	1.36	-	-
84	28.4	33.8	7.95	0.57	7.63	61	8.30	39	0.46	-	6.98	11	62
87	28.7	33.0	8.02	0.55	7.88	75	8.54	25	0.45	-	9.12	10	44
83	27.5	33.2	7.87	0.48	7.87	100	-	-	-	-	1.46	-	-
90	26.4	31.7	7.82	0.48	7.82	100	-	-	-	-	1.08	6	37
77	25.5	33.1	7.84	0.54	7.44	52	8.21	48	0.42	-	6.56	4	-
83	27.5	33.2	7.98	0.47	7.98	100	-	-	-	-	2.54	4	-
79	27.9	35.1	8.46	0.63	8.19	70	9.08	30	0.45	-	7.18	-	-
72	26.2	36.5	8.88	0.72	8.29	43	9.25	57	-	0.50	5.39	43	37
79	27.9	35.1	8.74	0.62	8.24	42	9.13	58	-	0.43	6.24	39	-
74	25.8	35.1	8.88	0.71	8.35	45	9.31	55	-	0.49	3.69	-	-
71	27.0	38.1	8.82	0.63	8.44	55	9.29	45	0.46	-	4.53	52	29
74	27.9	37.6	8.79	0.64	8.38	50	9.19	50	0.48	-	1.64	40	-
74	26.8	37.6	8.84	0.63	8.44	55	9.02	45	0.41	-	8.85	-	-
78	28.6	36.9	8.77	0.55	8.50	61	9.21	39	0.43	-	3.43	-	-
73	27.9	38.0	8.95	0.63	8.53	48	9.32	52	-	0.44	3.10	-	-
79	27.8	35.1	8.58	0.68	8.25	55	9.08	45	0.42	-	10.61	-	-
89	28.1	31.8	8.60	0.63	8.31	50	8.97	50	0.44	-	3.60	-	7
73	26.8	36.9	8.46	0.62	7.91	34	8.75	66	-	0.43	1.33	12	23
70	29.5	42.0	8.90	0.59	8.28	11	8.98	89	-	0.49	4.94	20	50
80	27.0	33.6	8.58	0.53	8.00	22	8.75	78	-	0.46	3.06	10	73
63	26.2	42.0	8.77	0.54	8.24	30	9.00	70	-	0.47	11.59	-	-
80	30.0	37.2	8.33	0.51	8.13	71	8.81	29	0.43	-	4.21	7	93
-	26.5	-	8.55	0.55	8.38	71	8.92	29	0.45	-	3.54	5	61

CHRONIC HEPATITIS		DATE	Hb	R b c	Vol %
<p>No. 38: M. L. G. W. b. 4/1-76. C. no. 1477/45, 26/10-45—27/12-45. Jaundice two days before adm. Tk. +++ at admission and till 9/4-46 when last observed. 27/10: R. f.: 0.26/0.42, 14/12: A/G.: 3.8/3.0, 7/4: A/G.: 4.4/4.2. T.: 8/12—27/12: Niacin 0.10 i. m. daily.</p>		27/10	11.7	4.14	40
		29/10	12.2	4.34	39
		5/11	13.3	4.68	41
		12/11	12.0	4.19	37
		20/11	12.4	4.60	39
		26/11	12.4	4.40	39
		5/12	12.3	4.40	36
		14/12	12.3	4.00	36
		27/12	13.3	4.40	38
		15/3	13.4	4.92	38
		9/4	12.8	4.50	37
<p>No. 39: I. S. W. b. 10/4-94. C. no. 1092/45, 29/5-45—6/10-45. Jaundice 3 months previously, for 2 weeks. Jaundice again 2 weeks before adm. when the liver was felt 2 cm. below c. m. 30/5: R. f.: 0.26/0.42, 7/7: A/G. 3.0/3.6. Tk. +++ during the stay in hospital. T.: 18/7: Liver-extract 100 cc. i. m.</p>		30/5	11.7	4.44	37
		6/6	12.2	4.54	38
		13/6	10.9	4.56	33
		19/6	9.5	4.10	30
		26/6	12.2	4.68	37
		29/6	13.1	4.88	39
		4/7	12.3	4.58	37
		11/7	9.8	3.62	29
		18/7	9.3	3.40	26
		23/7	9.1	3.20	25
		28/7	9.3	3.30	25
		1/8	8.8	3.10	24
		9/8	8.3	2.76	22
		16/8	7.6	2.50	22
		25/8	8.1	2.74	21
		3/9	7.9	2.94	23
		29/9	10.1	4.04	30
<p>No. 40: S. B. W. b. 30/6-97. C. no. 287/46, 3/8-45—9/3-46. Jaundice for 5 months, ascites for one week before adm. During stay in hospital repeated paracentesis. 17/2: General oedema and pleural effusion. 9/3: Started with milk diet. anasarca disappeared and conditions grew gradually better. At control april 1948 no jaundice but persistent macrocytosis, hypoprotrombinaemia, and positive Takatas reaction. 3/8: R. f.: 0.20/0.36, 26/10: A/G.: 2/3.8, 4/1: A/G.: 1.8/3.9, 31/1: A/G.: 1.7/3.6, 16/2: A/G.: 2/4.3. T.: 30/8—9/9: Aneurin 0.025 g. daily i. m. 4/12—30/12: Niacin 0.10 daily i. m.</p>		3/8	13.2	5.22	-
		13/8	13.8	4.62	41
		17/8	-	-	-
		22/8	13.4	4.72	42
		28/8	13.2	4.64	42
		6/9	12.7	4.64	41
		12/9	13.0	4.68	40
		20/9	13.5	5.00	35
		27/9	14.5	4.80	44
		4/10	13.0	4.60	34
		11/10	12.7	4.60	36
		26/10	13.8	4.86	42
		3/11	13.5	4.96	40
		10/11	14.5	5.20	42
		17/11	14.0	4.84	36
		24/11	13.8	4.92	40
		1/12	12.5	4.52	39
		8/12	13.4	4.96	39
		22/12	12.4	4.72	34
		30/12	12.3	4.42	32
<p>No. 41: M. G. W. b. 6/8-90. C. no. 1301/45, 30/10-45—21/11-45. Jaundice for 7 months before adm. when liver was felt 6 cm. b. c. m. Tk. +++ during the stay and at control 19/6-46. 31/10: R. f.: 0.28/0.42.</p>		31/10	11.4	3.80	36
		6/11	11.6	3.82	37
		13/11	12.0	4.14	37
		20/11	12.2	4.21	37

Corp vol	MCH	MCC	MD	s	m <sub>1</sub>	N <sub>1</sub> %	m <sub>r</sub>	N <sub>r</sub> %	s <sub>1</sub>	s <sub>r</sub>	$\chi^2$	I	S	R
97	28.2	29.2	8.89	0.74	8.26	42	9.31	58	-	0.47	5.57	20	18	-
90	28.2	31.2	8.76	0.66	8.38	56	9.28	44	0.43	-	4.57	-	-	-
88	28.4	32.4	8.97	0.62	8.48	44	9.38	56	-	0.44	1.65	74	25	-
89	28.6	32.4	9.03	0.57	8.52	24	9.25	76	-	0.45	2.58	80	12	-
85	27.0	31.8	8.81	0.57	8.35	41	9.13	59	-	0.44	6.12	39	8	-
89	28.2	31.7	9.16	0.52	8.40	12	9.25	88	-	0.45	0.22	28	26	-
82	28.0	34.2	8.93	0.55	8.17	16	9.05	84	-	0.48	3.79	19	25	-
90	30.8	34.3	9.02	0.59	8.38	29	9.25	71	-	0.44	1.18	20	-	-
87	30.3	35.0	8.81	0.66	8.38	51	9.27	49	0.44	-	2.54	20	22	-
77	27.2	35.4	9.01	0.55	8.13	12	9.13	88	-	0.46	0.73	6	38	-
82	28.4	34.5	8.53	0.60	8.00	30	8.83	70	Graphic	-	3.63	14	44	-
83	26.4	31.6	7.55	0.56	7.38	75	8.08	25	0.48	-	1.33	28	20	-
84	26.9	32.1	7.64	0.61	7.38	76	8.30	27	0.49	-	2.90	34	37	-
72	23.9	33.0	7.83	0.49	7.63	75	8.34	25	0.44	-	0.99	46	38	-
73	23.2	31.7	7.49	0.53	7.31	77	7.97	23	0.48	-	2.55	44	39	-
79	26.1	33.0	7.37	0.49	7.31	88	8.12	12	0.46	-	0.51	62	42	-
80	26.8	33.6	7.37	0.50	7.25	86	8.04	14	0.43	-	4.85	58	-	-
81	26.9	33.3	7.44	0.54	7.25	72	8.03	28	0.44	-	1.43	54	-	-
80	27.0	33.8	7.62	0.48	7.62	100	-	-	-	-	0.63	40	35	-
77	27.4	35.7	7.61	0.54	7.50	91	8.38	9	0.50	-	0.32	27	95	-
78	28.4	36.5	7.52	0.57	7.31	74	8.18	26	0.45	-	0.83	20	-	-
76	28.2	37.1	7.43	0.56	7.38	92	8.26	8	0.51	-	3.79	-	-	-
77	28.4	36.7	7.53	0.52	7.44	91	8.39	9	0.47	-	1.29	17	-	-
80	30.1	37.8	7.64	0.52	7.50	82	8.31	18	0.44	-	1.52	-	-	-
88	30.4	34.6	7.78	0.47	7.78	100	-	-	-	-	1.12	-	74	-
77	29.6	38.6	7.45	0.52	7.38	86	7.88	14	0.46	-	1.16	6	-	-
78	26.9	34.4	7.61	0.53	7.50	84	8.17	16	0.48	-	1.27	6	90	-
74	25.1	33.6	7.58	0.49	7.50	82	8.08	18	0.48	-	5.01	6	74	-
-	25.5	-	8.98	0.72	8.38	45	9.45	55	0.41	-	1.46	69	18	-
89	30.0	33.5	8.85	0.72	8.38	54	9.42	46	0.48	-	2.99	85	19	-
-	-	-	8.92	0.63	8.50	43	9.31	57	-	0.45	1.11	-	-	-
89	28.4	31.9	8.78	0.71	8.25	48	9.20	52	0.48	-	0.94	38	16	-
91	28.5	31.4	8.97	0.72	8.32	39	9.38	61	-	0.51	3.46	36	-	-
88	27.4	31.0	9.05	0.66	8.63	45	9.44	55	-	0.48	2.04	17	16	-
86	27.8	32.5	8.93	0.58	8.32	22	9.13	78	-	0.49	1.79	-	-	-
70	27.0	38.6	8.83	0.53	8.31	27	9.00	73	-	0.45	1.57	-	-	-
92	30.2	33.0	9.07	0.61	8.42	27	9.32	73	-	0.47	0.41	39	20	-
74	28.3	38.2	8.80	0.55	8.44	63	9.24	37	0.48	-	1.60	-	-	-
78	27.6	35.2	8.55	0.57	8.25	60	9.05	40	0.48	-	3.35	16	21	-
87	28.4	32.8	8.84	0.65	8.25	37	9.19	63	-	0.44	2.61	16	-	-
81	27.2	33.7	8.83	0.55	8.54	30	9.13	70	-	0.44	8.07	16	-	-
81	27.9	34.5	8.82	0.60	8.31	27	9.00	73	-	0.51	1.03	21	14	-
75	28.9	39.0	8.79	0.61	8.35	47	9.19	53	-	0.48	1.27	18	23	-
81	28.1	34.5	8.79	0.58	8.25	35	9.08	65	-	0.48	2.62	-	-	-
86	27.6	32.0	8.59	0.52	8.25	55	9.02	45	0.40	-	5.23	-	-	-
79	27.0	34.4	8.64	0.61	8.19	51	9.08	50	0.43	-	1.07	17	49	-
72	26.3	36.4	8.49	0.62	8.25	70	9.08	30	0.48	-	3.03	12	-	-
73	28.8	38.4	8.31	0.48	8.31	100	-	-	-	-	2.93	14	-	-
95	30.0	30.8	9.06	0.60	8.51	37	9.38	63	-	0.45	3.20	16	11	-
97	30.4	31.4	8.83	0.65	8.38	55	9.33	45	0.43	-	0.86	14	10	-
90	29.0	32.3	8.80	0.65	8.38	50	9.25	50	-	0.47	3.95	11	14	-
88	29.0	33.0	8.91	0.65	8.46	48	9.30	52	-	0.51	2.64	9	9	-

CHRONIC HEPATITIS		DATE	Hb	R b c	Vol %
<p>No. 42: S. S. W. b. 27/5-93. C. no. 1303/44, 27/5-44—10/7-44, 23/9-44—9/12-44. Jaundice of varying intensity for 7 months before adm. when liver was palpable 9 cm. below c. m. Died in coma hepaticum a month after discharge. R. f.: 9/6: 0.22/0.42, 27/6: R. f.: 0.26/0.44, 23/10: R. f.: 0.30/0.44. T.: 30/5—16/6: Lactoflavin 0.005 i. m. daily.</p>		30/5	12.3	4.02	32
		10/6	-	-	-
		14/6	11.6	4.22	31
		23/6	-	-	-
		30/6	11.1	3.70	31
		3/7	-	-	-
		10/7	-	-	-
		27/9	11.3	4.20	30
		9/10	-	-	-
		16/10	10.2	3.82	30
		1/11	-	-	-
		11/11	10.7	3.74	30
		25/11	11.2	3.84	31
		2/12	12.6	4.60	34
<p>No. 43: K. B. W. b. 22/7-84. C. no. 549/46, 2/3-46—9/5-46. No previous illness till January 1945 when jaundice. Treated in another hospital some weeks. Sub-icteric at time of discharge. Remained sub-icteric, but otherwise well till 2 months before admission. Tk. +++ repeatedly. 4/3: R.: 0.7%. 4/3: R. f.: 0.26/0.44, 4/3: A/G.: 2.8/4.5, 4/5: A/G.: 3.5/4.2. T.: 6/3—22/3: Niacin 0.10 i. m. daily. 11/4—21/4: Niacin 0.10 i. m. daily. 29/4—7.5: Nicotin 0.05 i. m. daily. a</p>		4/3	13.0	4.96	39
		15/3	11.6	3.92	32
		22/3	12.3	4.20	34
		30/3	11.8	4.22	32
		6/4	12.6	4.10	34
		13/4	12.4	4.32	33
		20/4	12.7	4.50	35
		26/4	13.5	4.74	35
		2/5	12.4	4.34	33
		10/5	12.4	4.50	33
		22/5	12.7	4.60	33
		31/5	13.0	4.64	-
<p>No. 44: E. L. H. W. b. 20/3-03. C. no. 1429/45, 8/11-45—20/12-45. Jaundice July 1943. Explor. laparotomy: Chronic hepatitis. Admitted in hospital for treatment for spondylitis. Tk. +++ 9/11: R. f.: 0.28/0.42, 13/12: A/G.: 3.8/4.1. R.: 0.5%. T.: 0.</p>		9/11	9.9	3.74	32
		16/11	9.7	3.78	32
		23/11	10.8	3.64	32
		30/11	10.4	4.02	32
		8/12	9.3	3.56	30
<p>No. 45: D. H. W. b. 10/4-86. C. no. 905/45, 21/6-45—18/8-45. Dyspepsia since 1934. Jaundice 3 weeks before adm. At adm. also slight oedema. Tk. ++++. 22/6: R. f.: 0.22/0.38. 18/8 rapid developing coma hepaticum. Mors. No autopsy.</p>		22/6	11.3	4.30	35
		2/7	11.2	4.20	35
		17/7	10.5	3.76	32
		24/7	10.5	3.96	31
		31/7	10.9	4.32	33
		9/8	10.2	4.00	31
<p>No. 46: E. M. F. W. b. 13/10-71. C. no. 552/45, 22/3-45—19/4-45. Cholecystitis 1924. 20/1-45: Pneumonia. Directly afterwards jaundice. 2 weeks before admission: oedema and ascites. Her condition at admission was poor and grew gradually worse. Mors. No autopsy. Tk. ++++. R. f.: 23/3: 0.32/0.44. T.: 0.</p>		23/3	11.8	4.02	30
		26/3	-	-	-
		31/3	12.6	4.42	35
		7/4	10.4	3.48	29
		13/4	11.8	4.32	30
		20/4	11.1	3.95	34
		1/5	11.7	4.25	34
<p>No. 47: N. E. W. b. 16/11-80. C. no. 1430/45, 19/10-45—20/12-45. 7 months before adm. malaise, jaundice. At adm. jaundice and anasarca. 20/12: Coma hepaticum. Mors. Autopsy: Chronic hepatitis. Tk. ++++. 20/12: R. f.: 0.26/0.44. T.: Hepto B (Liver extract with vit. B) 5 ccm. weekly. 2/10—20/12: Vit. K 0.01 i. m. weekly.</p>		20/10	12.0	4.62	38
		27/10	12.3	4.14	38
		3/11	12.4	4.58	38
		10/11	13.2	4.78	37
		17/11	12.6	4.66	35
		24/11	11.8	4.22	36
		1/12	13.1	4.52	35
		-	-	-	-
<p>No. 48: O. H. J. W. b. 23/2-77. C. no. 568/46, 7/3-46—14/5-46. November 1943: Jaundice. In another hospital for one month. Since then periodically jaundiced. June 1945: ascites. August 1945: General oedema. 12/5: Haematemesis. Mors. No autopsy. 8/3: R. f.: 0.28/0.46. T.: 0.</p>		8/3	11.3	4.00	33
		16/3	10.5	3.72	30
		23/3	10.5	3.92	29
		30/3	11.7	4.48	30
		6/4	10.9	3.96	30
		20/4	9.9	3.42	29
		27/4	10.1	3.50	28
		4/5	10.6	3.52	28
		11/5	11.7	4.16	31
		13/5	11.2	3.60	31
		-	-	-	-

Corp vol	MCH	MCC	MD	s	m <sub>1</sub>	N <sub>1</sub> %	m <sub>2</sub>	N <sub>2</sub> %	s <sub>1</sub>	s <sub>2</sub>	$\chi^2$	I	S R.
79	30.6	38.2	8.66	0.65	8.25	56	9.12	44	0.51	-	1.81	55	70
-	-	-	8.74	0.66	8.23	36	9.00	64	-	0.50	3.95	60	52
74	27.5	37.5	8.61	0.65	8.22	37	8.88	63	-	0.49	2.52	49	74
-	-	-	8.51	0.62	8.13	53	8.97	47	0.42	-	4.63	46	76
84	30.0	35.8	8.38	0.63	8.00	54	8.85	46	0.43	-	1.28	-	-
-	-	-	8.48	0.62	7.73	34	8.75	66	-	0.50	8.13	32	73
-	-	-	8.77	0.60	8.38	53	9.23	47	0.50	-	1.17	45	85
72	27.0	37.7	8.56	0.58	8.17	35	8.81	65	-	0.53	0.48	21	90
-	-	-	8.69	0.58	8.13	18	8.81	82	-	0.56	0.82	21	95
79	26.7	34.0	8.37	0.63	7.81	32	8.63	68	-	0.48	9.28	31	113
-	-	-	8.38	0.58	7.76	24	8.50	76	-	0.48	4.12	-	-
80	28.6	35.7	8.53	0.62	8.13	55	8.97	45	0.46	-	1.08	41	74
81	29.2	36.2	8.40	0.58	7.75	15	8.50	85	Graphic	-	3.49	40	64
74	27.4	37.1	8.24	0.64	7.76	40	8.56	60	-	0.52	1.14	16	42
79	26.2	33.2	8.38	0.52	7.81	33	8.50	67	-	0.48	2.69	90	57
82	29.6	36.3	8.39	0.56	7.88	50	8.88	50	0.47	-	8.25	63	39
81	29.3	36.2	7.84	0.56	7.75	86	8.57	14	0.48	-	0.69	26	30
76	28.0	37.0	8.28	0.65	7.91	47	8.69	53	-	0.48	4.68	40	82
83	30.7	37.0	8.57	0.57	7.93	24	8.75	76	-	0.43	0.43	-	-
77	28.8	37.5	8.14	0.73	7.88	50	8.75	50	0.48	-	1.32	30	47
78	28.3	36.3	7.95	0.59	7.75	80	8.69	20	0.48	-	0.80	-	35
74	28.6	38.5	8.15	0.65	7.68	47	8.56	53	-	0.45	0.46	25	17
76	28.6	37.6	8.21	0.56	7.74	26	8.38	74	-	0.50	6.44	26	40
73	27.6	37.6	8.32	0.63	7.52	20	8.50	80	-	0.51	1.83	18	-
72	27.6	38.4	8.04	0.56	7.45	22	8.19	78	-	0.46	1.74	16	-
-	28.1	-	8.13	0.56	7.88	30	8.30	71	-	0.49	7.90	12	24
86	26.4	30.8	8.12	0.65	7.59	40	8.50	60	-	0.48	3.48	30	25
85	25.8	30.3	8.22	0.61	7.86	52	8.63	48	-	0.43	3.70	-	-
88	29.7	33.8	8.23	0.61	7.50	24	8.50	76	-	0.47	1.44	-	-
80	26.9	32.5	8.05	0.74	7.55	55	8.66	45	0.54	-	2.89	-	-
84	26.1	31.0	8.00	0.72	7.56	40	8.38	60	0.47	-	2.97	-	49
82	26.3	32.3	8.20	0.67	7.74	47	8.63	53	-	0.43	7.46	50	13
83	26.8	32.0	7.96	0.64	7.69	60	8.47	40	0.45	-	4.53	33	12
85	27.9	32.8	8.04	0.58	7.75	72	8.63	28	0.48	-	0.30	42	12
79	26.5	33.8	8.09	0.69	7.63	48	8.56	52	-	0.42	2.84	-	-
77	25.2	33.0	8.20	0.67	7.74	46	8.63	54	-	0.43	1.36	60	-
78	25.5	33.0	8.17	0.59	7.68	41	8.50	59	-	0.48	1.07	-	-
75	29.4	39.4	7.95	0.60	7.63	65	8.41	35	0.48	-	3.19	33	15
-	-	-	8.18	0.57	7.83	58	8.59	42	0.48	-	11.80	19	-
79	28.6	36.1	8.42	0.60	8.06	60	8.88	40	0.47	-	0.56	-	-
83	30.0	35.8	8.16	0.65	7.91	42	8.75	60	-	0.48	3.21	18	14
70	27.4	39.4	8.19	0.59	7.88	65	8.66	35	0.47	-	1.12	-	-
86	27.1	32.6	8.02	0.56	7.88	84	8.66	16	0.47	-	0.79	-	-
80	27.6	34.6	7.92	0.63	7.50	50	8.33	50	0.45	-	0.51	-	18
-	-	-	7.82	0.53	7.75	75	8.44	25	0.47	-	1.32	33	-
83	26.0	31.5	8.51	0.57	8.04	44	8.88	56	-	0.42	2.33	54	37
92	29.8	32.4	8.48	0.54	8.12	41	8.75	59	-	0.48	3.42	-	-
83	27.0	32.6	8.46	0.63	8.00	53	8.94	47	0.45	-	2.02	48	-
78	27.6	35.7	8.56	0.67	8.04	46	9.00	54	-	0.48	1.99	19	13
75	27.1	36.0	8.58	0.70	8.13	58	9.11	42	0.48	-	1.50	-	-
85	28.0	32.8	8.71	0.75	8.25	55	9.32	45	0.48	-	0.80	20	14
78	29.0	37.5	8.60	0.86	?	?	?	?	?	?	-	24	10
83	28.2	34.2	8.87	0.70	8.40	51	9.38	49	-	0.47	1.72	26	32
81	28.2	35.0	8.75	0.58	8.38	44	9.06	56	0.44	-	4.71	-	-
74	26.8	36.2	8.57	0.54	8.38	68	9.05	32	0.44	-	8.98	-	18
67	26.1	38.8	8.92	0.60	8.50	43	9.33	57	0.44	-	6.84	-	-
76	27.4	36.3	8.75	0.68	8.22	41	9.13	59	-	0.42	2.00	15	23
85	28.9	34.0	8.39	0.62	7.93	45	8.75	55	-	0.44	4.68	-	10
80	28.8	36.2	8.51	0.65	8.13	50	8.95	50	0.45	-	2.31	-	-
79	30.1	37.8	8.50	0.64	8.07	50	8.95	50	0.46	-	5.23	-	6
75	28.1	37.7	8.75	0.69	8.19	42	9.13	58	-	0.47	2.41	-	-
86	31.1	36.1	8.94	0.66	8.17	23	9.13	77	-	0.50	1.29	-	-

CHRONIC HEPATITIS		DATE	Hb	R. b c	Vol %
No. 49: K. R. M. b. 12/6-92. C. no. R. H. 10375/47, 22/3-47—16/4-47. January 1946: Pericarditis. Mars 1946: Oedema and ascites. At adm.: Jaundice. Died suddenly 16/4. Autopsy: Cirrhosis hepatis. Pericarditis chronica. Tk. + + +. 24/3: A/G.: 4.2/1.73. T.: 30/3—16/4; Folic acid 0.05 x 3 daily.		24/3 27/3 29/3 31/3 2/4 5/4 8/4 10/4 12/4	16.3 15.7 16.0 15.7 16.0 16.0 15.2 15.4 15.4	5.05 5.36 5.22 5.05 5.06 5.04 4.94 4.93 4.93	47 49 44 46 47 49 46 46 50
No. 50: A. H. M. b. 18/4-75. C. no. 629/46, 25/3-46—16/4-46, 16/5-46—27/5-46. 14/3-46: Suddenly jaundiced without other symptoms. Grew gradually worse and died 27/5. Autopsy: Cancer caput pancreat. + Chronic hepatitis. Tk.: + + +. 26/3: A/G.: 4.8/1.7. R. f.: 0.24/0.38. T.: 6/4—16/4: Lactoflavin 0.005 i. m. daily.		26/3 1/4 8/4 23/4 30/4 7/5 14/5	11.8 10.6 10.2 11.3 10.2 11.2 11.1	4.24 3.72 3.60 3.90 3.64 3.70 4.06	32 27 27 26 27 27 27
No. 51: S. K. W. b. 9/4-96. C. no. 926/44, 12/7-44—28/8-44. Jaundice since may 1944. Died 28/8: Autopsy: Chron. hepatitis.		13/7	11.0	4.04	32
No. 52: J. M. M. b. 29/1-92. C. no. 343/45, 31/3-45—2/4-45. Cirrhosis hepatis since 1933. R. f.: 0.30/0.46, A/G.: 3.8/2.8.		30/1	8.7	3.66	30
No. 53: B. J. W. b. 6/7-01. C. no. 408/45, 5/4-45—4/5-45. Jaund. dec. 44. Autopsy: Cirrhosis. R. f.: 0.26/0.44. R.: 1.7 %.		6/4	12.8	4.56	37
No. 54: P. S. W. b. 3/7-82. C. no. 557/45, 7/5-45—22/5-45. Jaundiced 8 d. b. a. Died some month later. R. f.: 0.30/0.44.		4/5	13.9	5.02	40
No. 55: K. S. W. b. 10/1-64. C. no. 803/45, 13/3-45—4/5-45. Chronic hepatitis since mars 44. Tk. + + +. R. f.: 0.32/0.42.		14/3	10.5	3.74	32
No. 56: D. M. B. W. b. 29/10-72. C. no. 1452/45, 14/12-45—22/12-45. Autopsy: Cirrhosis. R. f.: 0.28/0.44. A/G.: 2.3/3.4.		15/12	11.2	3.92	33
No. 57: M. L. W. b. 16/4-80. C. no. 258/46, 4/2-46—5/3-46. Autum 1945: Hepatitis. Coma hepaticum 5/3-46. R. f.: 0.22/0.44. A/G.: 2/4.		5/2	11.0	3.80	32
No. 58: O. H. M. b. 20/4-85. Policlinic, 19/6-46. Hepatis chron. 1½ year. Died ½ year later. Tk. + + +. R. f.: 0.34/0.44.		19/6	13.4	4.76	39
CHOLECYSTITIS/CHOLELITHIASIS		16/7 23/7 24/7 25/7 28/7 2/8 7/8	13.3 13.3 12.7 12.4 12.4 12.2 12.4	4.78 4.88 4.70 4.54 4.52 4.76	39 38 39 35 36 37
No. 59: M. H. J. W. b. 15/4-10. C. no. 924/45, 14/7-45—25/8-45. 2 month bf. adm.: cholecystitis. New attack day before adm. 23/7: Operation: No gallstone. Tk.: ÷.		11/10 21/10 29/10 6/11 12/11 20/11 27/11 15/12	13.4 13.4 13.0 12.3 12.3 12.4 12.4 -	4.92 - 4.80 4.56 - 4.46 - -	41 - 39 37 37 36 -
No. 60: K. T. W. b. 13/3-94. C. no. 1395/43, 9/10-43—18/12-43. Repeatedly gallstone attacks last 6 weeks. 30/10: Operation: Stone in the common duct. Tk. ÷.		21/7 28/7 4/8 10/8 23/8	13.9 13.2 11.7 11.3 13.1	4.80 5.06 4.20 4.34 5.44	37 40 33 36 40
No. 61: F. J. W. b. 13/3-94. C. no. 944/45, 20/7-45—1/9-45. 13/6 and 21/6: Fever, and jaundice. Xray: Gallstone. T: O. Tk. —. R. f.: 0.28/0.42.					

Corp vol	MCH	MCC	MD	s	m <sub>l</sub>	N <sub>l</sub> %	m <sub>r</sub>	N <sub>r</sub> %	s <sub>l</sub>	s <sub>r</sub>	$\chi^2$	I. I.	S R
93	32.4	34.7	8.40	0.64	8.13	65	8.95	35	0.48	-	1.52	30	-
91	29.3	32.0	8.78	0.66	8.28	40	9.03	60	-	0.48	2.99	-	-
84	30.6	36.4	8.76	0.65	8.33	46	8.88	54	-	0.44	12.22	-	-
91	31.1	34.1	8.53	0.55	8.25	56	8.90	44	0.48	-	12.38	-	-
93	31.6	34.0	8.56	0.57	8.19	53	8.97	47	0.47	-	12.28	-	-
90	29.5	32.6	8.55	0.54	8.13	45	8.88	55	0.48	-	4.23	-	-
93	30.9	33.1	8.70	0.58	8.32	51	9.13	49	-	0.49	8.56	-	-
93	31.3	33.5	8.70	0.64	8.22	45	9.00	60	-	0.48	4.02	-	-
102	31.2	30.8	8.70	0.50	8.27	45	8.88	55	-	0.44	1.33	-	-
75	27.8	37.0	8.92	0.55	8.39	18	9.06	82	-	0.50	2.94	67	61
73	28.6	39.3	8.77	0.57	8.29	34	9.00	66	-	0.49	3.11	54	58
75	28.4	37.8	8.89	0.66	8.60	40	9.25	60	-	0.44	5.53	76	56
67	29.0	43.5	8.55	0.65	8.14	50	8.94	50	-	0.48	5.95	49	34
74	28.0	37.8	8.50	0.72	8.21	46	9.00	54	-	0.43	2.66	64	90
73	30.3	41.5	8.68	0.62	8.27	50	9.06	50	-	0.44	1.49	59	77
67	27.3	41.0	9.06	0.70	8.38	30	9.44	70	-	0.50	3.76	105	94
79	27.3	34.4	8.61	0.62	8.06	22	8.75	78	-	0.49	4.60	86	53
82	23.8	29.0	8.11	0.61	7.90	60	8.68	40	0.50	-	2.45	5	25
61	28.1	34.6	8.96	0.55	8.53	49	9.38	51	-	0.44	3.43	74	87
80	27.6	34.7	8.26	0.54	8.18	90	8.93	10	0.42	-	3.80	35	13
86	28.1	32.8	7.80	0.54	7.68	85	8.43	15	0.48	-	2.67	7	23
84	28.6	34.0	8.54	0.67	7.93	21	8.63	75	-	0.50	6.70	15	35
84	29.0	34.4	8.58	0.67	8.08	50	9.08	50	0.42	-	0.70	10	31
82	28.2	34.2	8.33	0.63	8.00	50	8.75	50	0.50	-	0.16	10	30
82	27.8	34.1	8.21	0.49	8.21	100	-	-	-	-	1.40	31	26
78	27.3	35.0	8.13	0.44	8.13	100	-	-	-	-	0.16	-	-
83	27.0	32.6	8.25	0.54	8.13	85	8.80	15	0.48	-	1.80	-	-
-	-	-	8.14	0.47	8.14	100	-	-	-	-	2.46	-	-
77	27.3	35.4	8.28	0.53	8.06	73	8.72	27	0.45	-	8.35	13	-
80	27.0	34.0	8.07	0.48	8.07	100	-	-	-	-	2.15	-	-
78	26.1	33.6	8.01	0.51	8.01	100	-	-	-	-	6.93	-	-
84	27.2	32.7	7.61	0.46	7.61	100	-	-	-	-	0.71	80	51
-	-	-	7.72	0.52	7.63	90	8.46	10	0.48	-	3.07	48	46
82	27.1	33.4	7.44	0.43	7.44	100	-	-	-	-	1.00	45	-
-	-	-	7.60	0.46	7.60	100	-	-	-	-	1.66	65	82
81	27.0	33.2	7.50	0.43	7.50	100	-	-	-	-	0.40	40	38
-	-	-	7.50	0.50	7.50	100	-	-	-	-	1.67	29	44
83	27.7	34.5	7.51	0.48	7.51	100	-	-	-	-	2.82	38	54
-	-	-	7.45	0.48	7.45	100	-	-	-	-	2.24	12	70
77	29.0	37.6	7.54	0.48	7.54	100	-	-	-	-	1.98	3	25
79	26.1	33.0	7.48	0.44	7.48	100	-	-	-	-	3.20	5	33
79	27.9	34.3	7.49	0.43	7.49	100	-	-	-	-	3.84	-	31
83	26.0	31.2	7.38	0.42	7.38	100	-	-	-	-	3.15	-	-
74	24.1	32.8	7.44	0.53	7.44	100	-	-	-	-	3.58	-	2



CHOLECYSTITIS/CHOLELITHIASIS		DATE	Hb.	R b c	Vol %
No. 62: J. J. W. b. 5/5-86. C. no. 474/44, 31/12-43—29/4-44. Attacks of gallstone for 20 years. Last weeks jaundice. Xray: Gallstone. 20/1: Cholecystectomy.		3/1	10.6	4.18	32
		12/1	-	-	-
		19/1	11.2	3.98	32
		26/1	-	-	-
		2/2	-	-	-
		9/2	11.2	4.02	32
		19/2	-	-	-
		23/2	10.8	3.76	31
		1/3	-	-	-
		8/3	-	-	-
		15/3	12.8	4.70	38
No. 63: A. R. W. b. 25/4-77. C. no. 1118/45, 14/7-45—13/10-45. Attacks of gallstone for 7 years. Day after admission: 2 gallstones pr. rectum. 22/8: Operation: Stone in the common duct.		16/7	12.7	4.14	-
		20/7	12.3	4.62	37
		27/7	13.5	4.84	40
		3/8	11.8	4.90	34
		10/8	11.4	4.46	35
		17/8	12.4	4.36	37
		25/8	10.5	3.70	29
		31/8	10.4	3.64	30
		6/9	9.4	3.42	29
		12/9	-	-	-
		20/9	-	-	-
No. 64: H. S. W. b. 30/7-70. C. no. 528/45, 27/12-44—15/5-45. Adm. in hospital for heart failure. 24/1: Cholecystitis. Xray: No shadow. T.: 0. Tk. —. R. f.: 0.30/0.44.		8/1	12.4	4.42	38
		9/1	13.3	5.20	42
		10/1	13.3	5.04	44
		11/1	13.3	4.80	44
		13.1	13.2	4.50	44
		15/1	12.7	4.74	42
		16/1	12.7	4.70	42
		19/1	12.7	4.65	43
No. 65: H. B. M. b. 28/3-64. C. no. 799/46, 4/3-46—1/7-46. Repeated attacks of gallstone since 1934. 6/5: Operation: Stone in the common duct. 5/3: R. f.: 0.30/0.46, 9/3: A/G.: 4/2.6, 24/5: A/G.: 4.9/2.2.		5/3	13.3	4.72	37
		16/3	12.3	4.64	34
		23/3	11.8	4.32	33
		29/3	11.8	4.22	33
		5/4	12.7	4.56	33
		24/4	11.1	4.14	29
		7/5	11.7	4.60	31
No. 66: B. V. W. b. 14/8-79. C. no. 971/45, 14/7-45—7/9-45. Repeated attacks of gallstone since 1940. Xray: No shadow. T.: 0.		16/7	14.1	5.02	-
		21/7	14.2	5.08	41
		28/7	13.1	5.02	41
		4/8	13.3	4.74	36
		11/8	13.8	5.08	41
No. 67: J. G. W. b. 19/7-91. C. no. 1228/44, 7/9-44—6/12-44. Cholecystitis 2 m. b., adm. 23/7: Op.: Stone in the common duct.		8/9	13.1	4.76	38
No. 68: H. J. M. b. 18/3-75. C. no. 1169/45, 10/10-45—24/10-45. Cholecystitis 14 days before adm. T.: 0. R. f.: 0.32/0.36.		11/10	12.8	4.30	36
No. 69: O. A. M. b. 25/10-06. C. no. 1367/45, 6/11-45—8/12-45. Strict. of com. duct p. op. 12/11: Choledocotomia. R. f.: 0.28/0.42.		7/11	12.4	4.62	32
No. 70: M. B. W. b. 18/3-89. C. no. 264/46, 18/2-46—7/3-46. Cholecystitis. 5/3: Cholecystectomy. 7/3: Mors (embolia?).		19/2	12.7	4.58	37
No. 71: R. H. W. b. 14/9-73. C. no. 505/46, 25/4-46—27/4-46. 13/12-46: Cholecystectomy. New attack with icterus. T.: 0.		5/3	12.2	4.52	33
No. 72: G. S. M. b. 19/10-70. C. no. 912/46, 8/5-46—31/7-46. Cholelithiasis last 4 years. 13/5: Cholecystectomy.		8/5	16.0	5.32	38

Corp vol	MCH	MCC	MD	s	m <sub>l</sub>	N <sub>l</sub> %	m <sub>r</sub>	N <sub>r</sub> %	s <sub>l</sub>	s <sub>r</sub>	$\chi^2$	I. I	S. R.
77	25.4	33.2	8.29	0.46	8.29	100	-	-	-	-	1.30	86	108
-	-	-	8.31	0.51	8.19	77	8.68	23	0.49	-	1.26	-	106
80	28.2	35.0	8.10	0.47	8.10	100	-	-	-	-	3.24	27	-
-	-	-	8.22	0.57	8.13	90	9.02	10	0.48	-	2.75	-	-
-	-	-	8.18	0.55	8.18	100	-	-	-	-	1.71	-	-
80	27.8	35.0	7.93	0.53	7.83	84	8.46	16	0.48	-	0.28	25	-
-	-	-	8.54	0.61	8.15	40	8.94	60	-	0.44	1.47	-	-
83	31.4	38.2	8.25	0.57	8.00	60	8.70	40	0.49	-	1.52	14	-
-	-	-	8.31	0.55	8.13	65	8.65	35	0.48	-	4.93	-	-
-	-	-	8.30	0.55	8.06	70	8.69	30	0.47	-	0.60	-	-
81	27.2	33.6	8.06	0.55	7.82	80	8.60	20	0.44	-	3.37	10	-
-	30.7	-	7.82	0.47	7.82	100	-	-	-	-	4.13	10	76
80	26.6	33.2	7.92	0.45	7.92	100	-	-	-	-	1.45	-	-
83	27.9	33.8	7.91	0.47	7.91	100	-	-	-	-	2.37	-	-
70	24.2	34.8	8.08	0.45	8.08	100	-	-	-	-	1.00	-	-
78	25.6	32.6	7.96	0.50	7.96	100	-	-	-	-	3.60	-	66
85	28.4	33.5	7.81	0.52	7.75	95	8.50	5	Graphic	-	0.64	-	-
78	28.4	36.2	8.00	0.50	8.00	100	-	-	-	-	0.71	-	-
83	28.6	34.7	7.99	0.57	7.88	80	8.63	20	0.48	-	1.63	-	-
85	27.4	32.4	8.26	0.52	8.06	65	8.64	35	0.48	-	5.32	-	-
-	-	-	8.15	0.58	8.00	80	8.78	20	0.47	-	2.38	-	-
-	-	-	7.95	0.43	7.95	100	-	-	-	-	4.51	-	-
81	28.0	32.6	8.03	0.45	8.03	100	-	-	-	-	1.35	15	56
81	26.6	31.7	7.95	0.51	7.95	100	-	-	-	-	1.38	-	-
88	26.4	30.2	7.91	0.46	7.91	100	-	-	-	-	2.05	-	-
92	27.6	30.2	7.97	0.46	7.97	100	-	-	-	-	1.60	-	-
98	29.4	30.0	7.97	0.44	7.97	100	-	-	-	-	2.37	23	68
89	26.8	30.3	7.95	0.51	7.90	95	8.68	5	Graphic	-	0.31	-	-
89	27.0	30.3	8.21	0.53	8.13	90	8.88	10	Graphic	-	0.86	-	64
92	27.4	29.6	7.99	0.45	7.99	100	-	-	-	-	1.29	11	46
78	28.2	36.0	7.99	0.47	7.99	100	-	-	-	-	2.82	15	88
73	26.5	36.1	8.17	0.61	7.88	70	8.65	30	0.48	-	0.30	-	79
76	27.3	35.8	8.02	0.48	8.02	100	-	-	-	-	2.89	-	-
78	28.0	35.8	8.20	0.51	8.08	85	8.85	15	0.48	-	0.78	9	65
73	27.8	38.4	7.95	0.45	7.95	100	-	-	-	-	3.16	10	43
70	26.8	38.2	7.75	0.44	7.75	100	-	-	-	-	1.55	12	-
67	25.4	37.6	8.11	0.44	8.11	100	-	-	-	-	1.15	16	62
-	28.1	-	8.06	0.44	8.06	100	-	-	-	-	1.48	5	50
81	28.0	34.6	8.18	0.45	8.18	100	-	-	-	-	0.18	-	-
82	26.1	32.0	8.02	0.47	8.02	100	-	-	-	-	8.89	-	-
76	28.0	37.0	8.10	0.46	8.10	100	-	-	-	-	0.96	-	-
80	27.2	33.7	8.08	0.46	8.08	100	-	-	-	-	0.31	-	35
80	27.5	34.2	7.56	0.47	7.56	100	-	-	-	-	0.15	15	73
88	28.8	32.8	7.45	0.48	7.45	100	-	-	-	-	0.34	44	34
69	26.8	38.8	7.94	0.43	7.94	100	-	-	-	-	0.62	26	54
81	27.7	34.3	7.88	0.51	7.75	92	8.71	8	0.49	-	0.25	25	40
73	27.0	37.0	7.93	0.50	7.93	100	-	-	-	-	0.39	32	100
71	30.1	42.0	7.70	0.50	7.70	100	-	-	-	-	1.22	30	24

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The abbreviations here employed are those adopted by the Quarterly Cumulative Index Medicus, Chicago: American Medical Association.

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FROM THE PATHOLOGIC DEPARTMENT OF THE INSTITUTE OF RAY TREATMENT,  
HELSINGFORS GENERAL HOSPITAL, HELSINGFORS, FINLAND.  
(CHIEF: PROF. IVAR WALLGREN, M.D.)

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## P R E F A C E.

During the last few years I. WALLGREN, in a number of works, has described his observations of finer cell structures examined with methods which evidently have been well suited to throw new light on many cytologic and also hematologic problems. He has examined and compared living blood and bone-marrow cells in dark-ground illumination and transmitted light on the heating table. During these observations he has touched on the hitherto unknown fate of the normoblast nucleus, which has been intimately associated with the question of the origin of platelets. This gave rise to the idea of trying to solve, with these methods, a half-century old, but as yet not finally solved, hematologic problem. The present work was started with this end in view and is based on observations carried out during the autumn of 1947 and the spring of 1948.

It is a great pleasure to me to offer my sincere thanks to my honoured chief and teacher, Professor IVAR WALLGREN, M.D., for the interest he has taken in my work. He has not only given me the idea, but also aided me with his great experience during the course of the observations, and through almost daily discussions and critical inspection of details he has contributed decisively to the production of this work.

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*Georg-Fredrik Saltzman.*

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## I. Introduction.

The question of the discovery of blood platelets has not been definitely settled. As it has not been established with certainty by whom they were first observed, the exact time of the discovery is also uncertain. In many quarters DONNÉ, 1842, is considered to be the first. He did probably observe platelets, but many facts indicate that he included among them elements which according to later investigators should not be referred to this group. ZIMMERMANN, in 1847, probably observed platelets, but he also seems to have drawn too broad limits. The same applies to a number of other investigators [SCHULTZE, RIESS (343), RANVIER, and others] during the next three decades.

Present-day investigators are agreed that platelets as a properly defined element according to the opinion now prevailing, were first described by HAYEM, 1878, and BIZZOZERO, 1882. The former should be regarded as the first; the latter's conception of platelets as a pre-formed constituent of the blood approached the results of present-day investigations, although it was subjected to sharp criticism during several decades.

It seems most appropriate to begin the historical survey of the literature with the epoch ushered in by the two above-mentioned authors. From that period it was for the first time possible for the investigators to furnish valuable contributions to the question of the origin of platelets, as from then on platelets could be distinguished from other platelet-like elements.

As platelets, however, have comparatively few characteristic features, the investigators of recent times have also encountered difficulties, whether the examination was done with stained

or unstained, native or fixed preparations. It seems highly probable that also recent contributions to the question of the origin of platelets are based on the observation of platelet-like bodies, in many cases artefacts. Faulty optics, unsatisfactory production of preparations and inconsideration of pathologic variations seem to be the reasons why the discussion on the origin of platelets has become one of the liveliest in the field of hematology. TOCANTINS follows the same line of thought when he says, »To know what is not a platelet is perhaps equally as important as to know what is a platelet». The border-line between platelets and pseudoplatelets must be sharply defined. Experiences have shown that this seemingly naive statement is justified. It has been found necessary in the present communication to revert to this question in different contexts.

## II. The history of the origin of platelets.

The literature on the origin of platelets is as extensive as it is varied. As it has already been reviewed by several investigators, [HITTMAYER (202, 203), ROSENTHAL, TOCANTINS, and others], it would seem appropriate in the present communication to refer extensively to these previous reviews. None of them, however, appear to be complete, except perhaps HITTMAYER's review of the literature from 1918—1938. As the problem of the origin of platelets seems now to approach its solution, I find it doubly justifiable to give, in all essentials, a review as complete as possible, of the literature on this long-lived debatable question, despite the fact that a small number of works have not been available to me.

Ever since HAYEM and BIZZAZZO in a conclusive manner demonstrated that platelets are one of the three formed constituents of the blood, contributions to the discussion on the origin of platelets have appeared continuously in the literature. Already these two pioneers expressed such widely differing opinions as to the nature of platelets that the problem became immediately the subject of intense study.

HAYEM, certainly, never advanced a definite view as to the origin of platelets, but he was on the whole the only spokesman for the theory that platelets, or hemoblasts as he called them, were precursors of the erythrocytes. Still in 1928 HAYEM (183) maintained this opinion.

Nor did BIZZAZZO express a definite view as to the origin. He considered, however, that the conception of platelets as elements produced from white or red blood corpuscles should be discarded. Thus, according to him, platelets were a pre-formed

constituent of the blood. He did not share HAYEM's opinion, but in accordance with HAYEM he seemed to find a relationship between platelets and the coagulation of the blood.

Before long one theory after the other was started on the origin of platelets. Practically all cells imaginable have been designated as mother cells of platelets. In the literature the white and red blood corpuscles have played a prominent part in this respect, whereas many authors also claim that platelets are products of precipitation or independent cells.

The most important theories will be described in the following in the chronologic order in which they were brought forth. It should be pointed out that the chronologic order has also been applied whenever several authors are mentioned in succession.

### 1. Leukocytic origin.

The »Dorpat School», lead by SCHMIDT, were inspired by BIZZOZERO's investigations, although they did not share his views. SCHMIDT's students, FEIERTAG and SLEVOGT, observed platelet-like bodies which would originate from »die rothen Körnerkugeln» (clumps of platelets?) which in their turn were considered to be transitional stages between white and red blood corpuscles. This theory was modified by the Dorpat investigators HEYL and RAUSCHENBACH who asserted that also white blood corpuscles were capable of producing platelets. WEIGERT agreed with the opinion of the Dorpat school under the belief that BIZZOZERO's interpretation was conditioned by traumatic artefacts. Although these works were published after those of HAYEM and BIZZOZERO, they are based on a conception which is so antiquated that it is difficult to judge the results obtained by these authors and to attach any considerable value to them.

The theory that platelets originate from the leukocytes predominated for about one decade after BIZZOZERO. This fact is principally due to those authors who before 1878 more or less unanimously advocated a leukocytic origin. (SCHULTZE, and others). Modern investigators are doubtful as to what these authors actually observed. Inefficient methods of examination and

perhaps also too deep faith in authority are the reasons why this theory predominated for a relatively long period.

In 1880—1890 several authors still upheld the view that platelets originate from leukocytes [HALLA, HLAVA, LÖWIT (267), LILIENFELD, WERNICKI, ZENKER], but in most of the works a degree of uncertainty may already be noticed. The theory was, indeed, modified by some authors. Whereas the cytoplasm of the white blood corpuscles had, throughout, been regarded as the original material, LILIENFELD attributed the origin of platelets to the nucleus of the leukocytes. WERNICKI, according to TOCANTINS, attributed the origin of platelets to the eosinophilic granulocytes.

The leukocytic theory has later reappeared sporadically without attracting many followers. HEILBER (190) supported it, but changed his opinion considerably within a short time (191). The theory was later favoured by VON DECASTELLO & KRJUKOFF, CAMUÑEZ & DEL PUERTO, STOCKINGER and STOCKINGER & MAASSEN. RIESS who already in 1872 favoured a leukocytic origin, was still in 1921 disinclined to renounce this theory.

Some authors in 1920—1930 pointed out similarities between platelets and leukocytes. Certain serologic (ROSENTHAL & FALKENHEIM) and chemical (ENDRES, ENDRES & HERGET) similarities, as well as similarities with regard to the cataphoretic velocity (ABRAMSON) need not conflict with the modern megakaryocytic theory. As is well known, the megakaryocytes and the leukocytes have a sufficient number of common properties to render the above-mentioned viewpoints applicable also to the former.

Finally, a great number of authors consider that white blood corpuscles, normally or vicariously, beside other cells, may contribute to the formation of platelets. These hypotheses will be mentioned in other contexts.

## 2. Platelets as products of precipitation.

Despite the fact that already BIZZAZERO described platelets in circulating blood, KLEBS, supported by WEITZ, started a theory according to which no platelets, normally, are present



in the blood vessels. They would arise from erythrocytes only when the blood is shed. SALVIOLI undertook an investigation of WELTI's experiments and did not find his arguments conclusive. LÖWIT (267) shared KLEBS' and WELTI's opinion that no platelets are normally present in circulating blood and that they arise as products of precipitation only when the blood leaves the blood stream. LÖWIT was not able to observe them in blood which had been collected direct in oil. He believed that the white blood corpuscles would give rise to at least the majority of platelets. Later WOOLDRIDGE, according to BÜRGER, expressed the same opinion. PIANESE and CESARIS DEMEL (82) also believed that platelets were products of precipitation formed directly from the blood-plasma; platelets would, however, be present in intact blood vessels. According to the latter author, the precipitation was associated with the development of the promegakaryocyte into a mature megakaryocyte.

A few other authors have held similar views. BUCKMASTER, BROCKBANK, MARINO [opposed by DEETJEN (96)] and SCHILLING considered that the circulating blood was devoid of platelets. STOCKINGER partly in co-operation with MAASSEN, regarded platelets as a kind of detritus-masses, but maintained that they are also present in circulating blood. As mentioned above, they considered that platelets were derived from leukocytic substance, among other substances. LEVY recently brought forth the hypothesis that «platelets are smaller or larger precipitations of fibrin around hematokonia, etc., in a clotting blood».

### 3. Erythrocytic origin.

With regard to the finer structures the difference between platelets and mature erythrocytes is considerable. In the mature red blood corpuscles there is no parallel to the granulation observed without difficulty in platelets, whereas the structure of the leukocytes is much more similar to that of platelets. Therefore, it seems rather strange that in the opinion of most hematologists the mature erythrocytes took the place of the leukocytes as the mother cells of platelets.

b. *Nucleoid origin.*

The theory according to which platelets are derived from the nuclei of the nucleated red blood corpuscles has played an important part in the discussions on the origin of platelets. True, it has hardly convinced the majority of investigators at any time, but has, on the other hand, been a persistently disturbing factor. In the absence of conclusive counterevidence it has been kept alive for more than 50 years and has, up to the present time, called for consideration by critical investigators, also those who have held decidedly different opinions about the origin of platelets.

The strongest argument for the theory is that it explains the fate of the nucleus of the normoblast. Around this argument are centered a number of works which support the theory with evidence based on morphological, comparative anatomical, chemical, experimental pathological and clinical observations. It would, undoubtedly, have been possible to give most of the observations interpretations which harmonize with other theories, but the fact remains that direct and conclusive counter-evidence is lacking.

Besides AFFANASIEW, also GIBSON and ENGEL, at an early date, upheld the view that platelets originate from the cell nucleus. The theory received only scant support until MAXIMOW and PAPPENHEIM almost simultaneously renewed this attempt at explaining the origin of platelets. Their opinions, however, differed considerably. MAXIMOW considered that the whole nucleus of the normoblast, when sufficiently mature, gave rise to platelets, whereas PAPPENHEIM proposed that the biggest part of the nucleus disintegrated intracellularly, and only a small remnant of the nucleus was extruded as a platelet. HIRSCHFELD favoured PAPPENHEIM's opinion. In a detailed communication PREISICH & HEIM give a fairly comprehensive account of their viewpoint which, on the whole, harmonized with MAXIMOW's. Platelets are found only in blood which contains non-nucleated red blood corpuscles, that is in mammalian blood. Platelets consist of nuclear substances or, at least, contain an abundance of them. In the interior of the erythrocytes

in stained blood preparations these authors claim to have observed platelets arranged like nuclei. HELBER (191), later, abandoned his conception of the leukocytes as the origin of true platelets and attributed it to the nucleus of the normoblast.

SCHILLING is the most energetic defender of the theory that platelets originate from the nucleus of the normoblast. In his two principal works (371, 373) he presents exhaustive and comprehensive, chiefly indirect, arguments. He claims, indeed, that he in native preparations examined in dark-ground illumination has been able to witness, in exceptional cases, the extrusion of the nucleus of the normoblast and its transformation into a platelet.

SCHILLING holds that normal circulating blood contains no, or possibly a few, platelets. According to him some of the red blood corpuscles are, normally, nucleated or contain remnants of a nucleus, but this nuclear substance is extruded rapidly and gives rise to platelets, when the blood leaves the blood vessels. In order to prevent this extrusion of nuclei, he applies a method of rapid fixation (373), by means of which he, so to speak, catches the platelets on the run, on their way out of the red blood corpuscle. He even claims to have observed them right inside some erythrocytes. Chemical and clinical observations are quoted as further evidence for the theory. SCHILLING realizes that his argument is not altogether conclusive, and he has become more cautious with years. Still in 1943, (382), however, he considered his opinion to be justified, so long as there are no decisive evidence for any other theory on the origin of platelets or the fate of the nucleus of the normoblast.

SCHILLING, however, is not the only author who has supported the theory after 1912. WINOGRADOW, in a case of pernicious anemia, and BOFINGER in a case of spotted fever, observed pictures which in their opinion indicated that platelets originate from nuclei of normoblasts. ARNDT, DUESBERG and MATHIS also uphold this view. VOIT & KEMPA have made chemical observations in favour of SCHILLING's theory. HEKMA regards platelets as a product of undefined nature, arisen through some kind of interaction between the nucleus and the cytoplasm of the normoblast.

During the last decade the theory has again become the focus of interest through ØRSKOV's investigations. According to ØRSKOV, whose theory is a modification of SCHILLING's, platelets consist of the nuclear membranes + some nuclear stroma. Platelets are formed in the bone marrow and are a normal constituent of the circulating blood. This opinion is based chiefly on studies of peripheral blood in cases of combined phenylhydrazine-lead poisoning, where according to ØRSKOV, all transitional stages from nuclei of normoblasts to platelets may be observed. He also presents other, more indirect, evidence. MARCUSSEN supports ØRSKOV's theory.

#### 4. Combined erythrocytic and leukocytic origin.

So far as I can find, MÜLLER was the first to advocate that platelets originate from red as well as white blood corpuscles. According to him, most platelets are derived from the red blood corpuscles. SCHWALBE, later, was the chief spokesman for this theory in a series of communications, partly in co-operation with SOLLEY. His strongest defence against other views was that he claimed to have observed formation of platelets in doubly ligated portions of blood vessels. He also regarded the erythrocytes as the chief mother cells of platelets. WEIDENREICH (478—483), SCHNEIDER, MARTELLI and RETTERER favoured this opinion, the first-mentioned author emphasizing strongly the erythrocytic origin. Also GRAWITZ advocated a double origin. He considered, however, that platelets originate from the nuclear substance of red and white blood corpuscles. VON BAUMGARTEN was more doubtful. He claimed to have observed platelet-like fragments of white as well as red blood corpuscles, but, nevertheless, he did not consider it to be proved that they were identical with true platelets. DEREWENKO was never able to demonstrate a relationship between red and white blood corpuscles on the one hand, and platelets on the other. CASTRONUOVO & SPIRITO, according to ROSENTHAL, considered that white and red blood corpuscles and also endothelial cells were capable of producing platelets.

## 5. Platelets as independent cells.

Already BIZZOZERO considered that platelets were elements independent of red and white blood corpuscles. His view received only scant consideration during a couple of decades. He was not, however, altogether without followers. SCHIMMELBUSCH (see also EBERTH & SCHIMMELBUSCH), LAKER, ASCHOFF (18), LAVDOVSKY and SACERDOTTI, in their investigations, made no findings indicating that platelets were produced by any other component of the circulating blood. DEERTJEN, in 1901, revived this theory in a modified form, according to which platelets were complete cells. He was supported in rapid succession by DEKHUYZEN, KOPSCH and ARGUTINSKY. Also BÜRKER, ROSIN & BIBERGEIL and ZURHELLE considered that platelets were independent of other elements of the blood. The French authors, LE SOURD & PAGNIEZ (420), ACHARD & AYNAUD and COURMONT & ANDRÉ, later, supported the theory of platelets as independent cells. The two last-mentioned authors who claimed to have observed an increase in the number of platelets *in vitro*, met with immediate opposition from VAQUEZ, ACHARD and MAYER.

The conception of platelets as cells was then forgotten for another decade. After that ROULET, PERRONCITO and VAN HERWERDEN appeared as its spokesmen. PERRONCITO even claimed to have observed platelets in mitotic division. ESTRADA, BÜRKER, SCHAEFFER and FONIO, still quite recently, regarded platelets as cells. Later, FONIO in co-operation with SCHWENDENER, spoke very cautiously on the subject, but seemed inclined to regard platelets as cells. PETERSEN is the only author of textbooks, who has favoured this opinion.

The authors of all these works refrain from expressing an opinion on the origin of platelets. They all confine themselves to opposing the idea of a relationship with other elements of the circulating blood. Many of the authors mentioned in this chapter have designated platelets as thrombocytes. This designation has later been used frequently also by authors who did not regard platelets as complete cells. As, however, the term »thrombocyte» is likely to cause misunderstanding, I have tried to avoid it.

## 6. Megakaryocytic origin.

According to the theory which now has the greatest number of followers, platelets originate from the megakaryocytes. Many investigators consider the battle already settled in favour of this theory. It is borne out by a multitude of evidence which, however, seems to be more or less indirect. J. H. WRIGHT is the originator of the theory and it bears his name in the literature.

It may perhaps be of some interest to mention a few authors who already before WRIGHT seem to have approached the same view. FOA & CARBONE, in 1889, observed in the spleen large cells in the protoplasm of which they appear to have noticed platelet-like bodies. CZERMACK, 1893, attributed the origin of platelets to »Keimzellen» which he considered probably identical with the megakaryocytes. He proposes that the nucleus as well as the protoplasm supplies the material. HEIDENHAIN, in 1894, believed that he observed zones in the centre of the megakaryocytes, of which at least the peripheral were able to disintegrate. He considered that the megakaryocytes were connected with the formation of blood. They were, however, incapable of producing either red or white blood corpuscles.

These publications, however, seem to be of merely historical interest. WRIGHT has scarcely been influenced by them in any noteworthy degree. His first two communications on this subject appeared in 1906. He based his theory chiefly on examinations of bone-marrow slides from different mammals and man. He observed in them megakaryocytes which were situated extravascularly; into the vessels, however, protruded pseudopods from which were constricted off portions of cytoplasm exactly resembling platelets. He also observed on the heating table protoplasmatic movements along the periphery of the megakaryocytes, which were identical with the movements seen along the edge of the platelets. He pointed out the fact that megakaryocytes and platelets occur parallel in the mammals (but not in other animals), that they develop simultaneously during fetal life, and finally, that certain parallels in regard to frequency may be observed in pathologic conditions.

WRIGHT's theory became immediately the object of great interest and control investigations on animals were started. BUNTING, SCHRIDDE and OGATA supported the theory emphatically on the basis of such investigations. Prominent authors, ASCHOFF, DOWNEY and NAEGELI, among others, supported already at an early date WRIGHT's opinion on the basis of their own investigations. CESARIS DEMEL (79) seems to have been the first Italian author to uphold WRIGHT's views, although he later modified his attitude. Also other authors (KEIBEL, BROWN, KLEIN) found the theory correct but questioned the possibilities of a parallel origin. Also OELHAFEN favoured the view, although he was doubtful on certain points. LE SOURD & PAGNIEZ (421), at an early date, abandoned their conception of platelets as independent cells in favour of WRIGHT's theory. WEIDENREICH (484) who had earlier claimed that platelets originate from red as well as white blood corpuscles, was also at an early date found among the followers of WRIGHT. There was, however, no lack of criticism. SCHWALBE (408) and DEETJEN (95) who considered that their theories were seriously threatened, could not adopt the new conception. Also ASKANAZY (24), VON GIERKE and STERNBERG, at first, rejected the theory. SCHWALBE, however, was disinclined to rule out altogether the possibility that some platelets may originate from the megakaryocytes.

Towards the end of the first world war and particularly after it an exceedingly great number of works were published in support of WRIGHT's theory. The extensive literature seems to be out of proportion to the modest contributions that after WRIGHT's appearance were made in support of his theory. This fact together with the difficulty in assessing the value of the results published, renders it difficult to make a clear and exhaustive survey. Some of the findings which in my opinion are the most important ones, may, however, be quoted briefly.

A number of authors (FOÀ, DI GUGLIELMO, WITTKOWER, HERZOG & ROSCHER, HARTMANN, BETANCÉS, FONTANA, ROTHERMEL, DE OLIVEIRA, KINGSLEY, ROHR, ROHR & KOLLER, DU BOIS, GALINOWSKI, MALAMOS, SCHENKER, DE LA FUENTE) described *typical so-called Wright pictures*, that is, observations in which they seemed to notice a phase of the pinching off of

platelets from the cytoplasm of megakaryocytes. These pictures were at first observed in slides, but were later seen also in smears. Already NAEGELI (298) claimed to have observed platelet-producing megakaryocytes in smears from peripheral blood from patients with myeloid leukemia, polycythemia, indeed, in exceptional cases also in the blood of patients with leukocytosis. KAZNELSON (230), later, observed Wright pictures in peripheral blood from a patient with hemolytic jaundice and also MINOT has published pictures of apparently platelet-producing megakaryocytes in smears of blood. As evidence in support of WRIGHT's theory, SÖDERSTRÖM and UNDRITZ & ROTHLIN, recently, pointed out the agglutinability characteristic of both megakaryocytes and platelets, with special emphasis on the important fact that true Wright pictures must not be confused with findings conditioned by temporary agglutination between these two elements.

Some years ago SCHLOSSHARDT & HEILMEYER on examination with the »Luminiscenzmikroskop» observed great similarities between platelets and megakaryocytes.

*Chemical similarities* between megakaryocytes and platelets have also been mentioned as evidence in support of the theory. In two works (434, 435), the latter in co-operation with HORSTMANN and HILSNITZ, STAHL, who was already a follower of WRIGHT, reported a specific reaction on iodine impregnation, common to megakaryocytes and platelets. According to VON ROKAY, however, the results of a control investigation did not indicate a common reaction to iodine. VASATURO, however, according to WHITBY & BRITTON, reached the same conclusions as STAHL and his collaborators. According to CORRADETTI, the granules of megakaryocytes and platelets react in a specific manner to hydrochloric acid. The similarity with regard to the stainability of the granules, which was emphasized particularly by FREY and ASKANAZY (26), should also be mentioned here. KATSANUMA points out in particular similarities with regard to oxidase granules and Altmann's granules. The common feature of all these more or less chemical works is that they bear out WRIGHT's theory and present remarkable arguments against SCHILLING's views.



ROSENTHAL & FALKENHEIM, BIANCHINI, GRÜNBAUM and TOCANTINS & STEWART interpreted *serologic similarities* between megakaryocytes and platelets as evidence of a megakaryocytic origin. Although the two first-mentioned authors did not obtain specific reactions, they thought that they were able to demonstrate that platelets and erythrocytes have nothing in common. Thus they considered that they were justified in disregarding the only theory which in their opinion may have competed, reasonably, with WRIGHT's theory. COLE, already in 1907, claimed, on the basis of serologic examinations, that every connection between platelets and red blood corpuscles may be ruled out. BEDSON, also on the basis of serologic examinations, doubted that platelets were related, genetically, to any of the other elements in peripheral blood, without entering into further discussion on the origin of platelets.

Experiments to check or to promote the formation of platelets *by means of poisons* have given further support to WRIGHT's theory. SEELIGER, partly in co-operation with GORKE, carried out experiments with pepton poisoning and made valuable findings in favour of WRIGHT's theory. In their investigations they noticed a simultaneous decrease and increase in the number of platelets and megakaryocytes. FIRKET made similar observations in his experiments with saponin poisoning, which he carried out partly in co-operation with CAMPOS. MEDLAR, also in co-operation with HORNBAKER and ORDWAY, obtained the same results as FIRKET in their experiments with saponin poisoning, and found further evidence in experimental poisoning with benzol. WEISKOTTEN & WYATT & GIBBS, in their experiments with benzol injections, also obtained results which harmonized with WRIGHT's theory. Finally, BERNARD, by injecting tar, was able to check the formation of leukocytes as well as erythrocytes without affecting the formation of platelets. TURCHINI & GVOZDIEVA, however, were not able to demonstrate a relationship between megakaryocytes and platelets in experimental injections with saponin, pilocarpine and pyridine.

EKERFORS, on administering neosalvarsan, and HADORN, on administration of sedormid in ordinary doses, produced dis-

tinct parallel changes in the number of megakaryocytes and platelets.

FABRICIUS-MØLLER has made a comprehensive study of *the effect of the roentgen rays* on the formation of platelets. He also observed distinct and parallel changes in the number of megakaryocytes and platelets. GUNN, on radiation with ultraviolet light waves, made similar observations. In studies of the proportion between the number of megakaryocytes and platelets in specific infections he found further evidence for WRIGHT's theory.

Italian hematologists have studied platelets in experimentally produced *asphyxia* in animals. BIANCHINI, LEGA and FRANCONI made observations which seemed to them to indicate a genetic relationship between megakaryocytes and platelets. PETRI, on the other hand, in a large series of experiments including, for instance, asphyxia and various arrangements for venesection, obtained results which made him disagree definitely with WRIGHT's theory. His arguments were criticised by FREY (154) and KAUFFMAN. They did not consider them to be acceptable reasons for rejecting WRIGHT's theory.

*Clinical investigations* have also furnished some fairly important evidence in support of WRIGHT's theory. Long before he supported the precipitation theory, CESARIS DEMEL (80), on the basis of studies on leukemia, described similarities between megakaryocytes and platelets. Also BACALOGLU & TUDORAN and ARNETH drew their conclusions on the basis of investigations on leukemia.

GLANZMANN, GÁSPAR, KNOLL, DALLA VOLTA and ZITZMANN have described cases where platelets and megakaryocytes decreased in number, or disappeared, simultaneously. The two first-mentioned authors regarded their cases as ordinary cases of essential thrombocytopenia (Werlhof's disease) and found in them evidence for WRIGHT's theory.

FRANK, however, noticed that the number of megakaryocytes need not at all be reduced in Werlhof's disease. On the contrary, these cells are as a rule exceptionally numerous. According to FRANK, this fact need by no means be used as an argument against WRIGHT's theory, as it had been for obvious rea-

sons. On the contrary, FRANK supported whole-heartedly WRIGHT's views, arguing that the megakaryocytes were abnormal and incapable of producing platelets in the normal manner. According to him, the increase in the number of megakaryocytes was purely compensatory. FRANKS's opinion was later corroborated by SEELIGER who found abnormal megakaryocytes in typical cases of Werlhof's disease. JEDLIČKA, ELLMER, GERLACH, SCHMINKE, SAMEK & ARCHI and LEITNER (258) on the basis of their own investigations, also supported FRANK's opinion.

KAZNELSON opposed the above-mentioned theories on the blood and bone-marrow findings in Werlhof's disease. He did not believe that the megakaryocytes were injured in essential thrombocytopenia but attributed the low number of platelets to an increased disintegration of platelets. FOERSTER held a similar view. This conception did not prevent KAZNELSON (237) from presenting one of the most important clinical facts in evidence of WRIGHT's theory. He studied a case where the formation of leukocytes and erythrocytes was completely checked. The platelet formation, however, was normal and the usual number of megakaryocytes was observed. BAAR and HEILMEYER, later, reported similar cases.

This is not the place to discuss in detail the different theories on the pathogenesis of essential thrombocytopenia. It may be established, however, that the three conceptions which favour megakaryocytopenia, megakaryocytopathy and increased disintegration of platelets, respectively, have one common feature that is of importance in this connection. They all bear out the assumption that platelets originate from the megakaryocytes.

CARTWRIGHT & CHUNG & CHANG, recently, in cases of kala-azar with pancytopenia, found a decrease in the number of platelets and simultaneously a reduced ability of the megakaryocytes to produce platelets.

Cases with an increased number of platelets have also been used as arguments for WRIGHT's theory. EPSTEIN & GOEDEL, HAMAGUCHI & AKAZAKI, UOTILA, REID, OWREN, LEITNER (257), SÖDERSTRÖM and MORTENSEN have reported cases with a marked increase in the number of platelets as well as megakaryocytes. This increase in the number of platelets and mega-

karyocytes has also been found to be usual in polycythemia vera, although in this disease they seldom reach the same extreme numbers as in the above-mentioned cases. It should be mentioned here, however, that an increase in the number of platelets without a corresponding increase of megakaryocytes has also been observed, particularly in patients with myeloid leukemia. In these cases, however, the increase in the number of platelets may have been conditioned by pseudoplatelets (BIRCH).

FREY (154), on examination of a large material comprising different diseases, noted a general consistency between the number of platelets and the number of active megakaryocytes. JÜRGENS & GRAUPNER observed obviously parallel pathologic changes in megakaryocytes and platelets.

GLANZMANN in cases of hereditary hemorrhagic thrombasthenia, observed large platelets, whereas some of the megakaryocytes showed areas the size of these platelets. Besides other changes in the blood, also HEGGLIN, quite recently, observed in a patient large platelets, whereas all the megakaryocytes which appeared to be ready to produce platelets, were seemingly divided into areas the size of the large platelets. It would seem rather far-fetched to regard these observations as conditioned by a coincidence.

*Slight modifications have been proposed.* WILLI and SCHENKER held that also the promegakaryocytes were capable of producing platelets. The former asserted, indeed, that they would produce even more platelets than the mature megakaryocytes.

Several authors found it difficult to combine WRIGHT's theory with their own observations that platelets on staining give reactions typical of the cell nucleus, a fact which has been pointed out, most recently, by VOIT and his collaborators, and SCHENKER. ROHR & KOLLER were also able to demonstrate nuclear substance in platelets and assumed that the nucleus of the megakaryocyte, together with the cytoplasm, would take part in the formation of platelets. Later, MALAMOS upheld this opinion. STORTI & STORTI, on the other hand, were not able to demonstrate nuclear substance in platelets. Another argument against WRIGHT's theory, the difficulty in recognizing typical

Wright pictures, was met by ROHR & KOLLER with the hypothesis that the megakaryocytes would break up into platelets with explosion-like rapidity.

MARCHESINI tried to combine the theories of a megakaryocytic and an erythrocytic origin. According to him platelets arise from disintegrating red blood corpuscles extruded from the megakaryocytes and phagocytosed previously by them.

Numerous other authors have also supported the theory that platelets originate from the megakaryocytes. I do not intend to discuss their works but will only mention the names BENECKE, GAZETTI, CASTRONUOVO, GAVIATI, SLAWIK, KLASCHEN, REITANO, DEMMER, SABIN, FERRATA, MORAWITZ, CLAESON, KRITSCHESKY & TSCHERIKOWER, RENTZ, VOLTERRA, MACKAY, BOCK, GÖRÖG, BRUGSCH, WAHLBERG, INTROZZI, BEMELMANS, NORDENSON (304), STORTI, JÜRGENS, FLEISCH-HACKER & WALTERSKIRCHEN, SCHULTEN, TOCANTINS, THADDEA & BAKALOS, JAPA, MOSSBERG, WISLOCKI & BUNTING & DEMPSEY, KAHRS and SCHRUMPF (392), all of whom displayed a positive attitude to WRIGHT's theory. It seems to me, however, that their works have scarcely added any significant contributions to the discussion on the origin of platelets.

Those few authors of textbooks, who have been unable to agree with WRIGHT's theory, [GRAWITZ, HIRSCHFELD (199), SCHILLING (380, 381), PETERSEN], have been mentioned elsewhere in this communication. BURNHAM refrained from expressing a definite view on the subject of the origin of platelets, but seemed most inclined to believe in an erythrocytic origin. The great majority regard the question of the origin of platelets as more or less finally solved through WRIGHT's theory [ROSIN, MEULENGRACHT & GRAM, FRANK (152), PINEY, NAEGELI (300), BUNTING (68), ROSENTHAL, GRAM, ROHR (349), KRACKE, OSGOOD, ARNETH (11), HEILMEYER, PINEY & WYARD, WINTROBE, BOYD, EHRSTRÖM, KIENLE, THADDEA, FOWLER, LEITNER (258), SCHRUMPF (391), WHITBY & BRITTON]. A few authors, HIRSCHFELD & HITTMAYER, TANNENBERG & FISCHER-WASELS and SCHULTEN (394, 395), speak with caution, but regard WRIGHT's theory as the most probable. NORDENSON, who in his early textbooks (305, 306) favoured WRIGHT's opinion, proposes in

his latest edition (306 a) that platelets are formed from reticulo-endothelial cells besides megakaryocytes.

Most authors consider that »the birthplace of platelets», to quote the expression used by FRANK, is the bone-marrow. Already WRIGHT, however, proposed that also the megakaryocytes of the spleen were capable of producing platelets. This opinion was later supported by BROWN, LE SOURD & PAGNIEZ (421), CESARIS DEMEL (81), FIRKET, ASCHOFF (23) and BOCK, among others, whereas BEDSON & JOHNSTON found no indications of this mode of formation, at least in normal cases. HOWELL & DONAHUE are the only authors who hold that the majority of platelets are derived from the megakaryocytes in the lungs. JORDAN (220) and FIDLAR & WATERS investigated this hypothesis and reached a different opinion. BÉTANCÈS claimed that platelets at the embryonic stage were produced by the megakaryocytes of the liver. MACKAY, without giving a closer definition of his opinion, considered that the bone-marrow, normally, was capable of producing platelets, but that also other organs may partake in the formation under conditions of an increased demand for platelets.

## 7. Various theories.

As a final group of hypotheses may be mentioned those which have received no noteworthy support in the literature. This, however, is the only feature they have in common. They should rather be regarded as a collection of extremes in the extensive and varied literature on this subject.

Some authors claim that also other cells besides megakaryocytes are capable of producing platelets. A number of authors consider that also »polykaryocytes» (osteoclasts?) besides the megakaryocytes, are mother cells of platelets [CESA BIANCHI, BIANCHINI, DI GUGLIELMO (173), MORONE, FONTANA, GANDOLFO, ROSENTHAL, DAMASHEK & MILLER]. The hypothesis was opposed by LAPIDARI and UNDRITZ & ROTHLIN. LUBARSCH believed that platelets were produced by many different cells, red blood corpuscles and megakaryocytes being the chief mother cells. BROWN emphasized that the megakaryocytes are the most

important mother cells of platelets but attributed the origin of platelets also to *hyperplastic endothelial cells* in the marrow and *mononuclear* and *transitional cells* in the marrow, spleen and blood. A similar view was held by SPADOLINI according to whom platelets originate from megakaryocytes, monocytes and endothelial cells, among other cells. He mentioned, in particular, the spleen as the source of the formation and emphasized that platelets contain nuclear substance. BUNTING (67) and KUCZYNSKI considered that *lymphocytes* and *plasma-cells* may take part in the formation of platelets, together with the megakaryocytes. BUNTING proposed that *all leukocytes* were capable of producing platelets, but only vicariously, however, in certain pathologic conditions. According to KOMOCKI, *all mononuclear leukocytes*, among which he seemed to include also the megakaryocytes, are capable of producing platelets. WOODCOCK attributes the origin of platelets to megakaryocytes and *monocytes*. MARTELLI seemed to be of the opinion that practically *all cells present in blood and bone-marrow*, including naturally the megakaryocytes, are capable of producing platelets. STOKINGER & MAASSEN believed that *all cells of the mesenchyma* were capable of producing platelets. KLEIN supported principally WRIGHT's theory, but attributed the origin of platelets also to a cell form described by him, »die Myelogenie». WATZKA considered that platelets are derived from *endothelial and reticular cells*, although he believed that the majority originate from the megakaryocytes. SAMPSON & KERR & SIMPSON, although on the whole supporting WRIGHT's theory, believed that they were able to demonstrate vicarious formation of platelets from *macrophages* in purpura in conjunction with septicemia. FABRIS considered that a *so-called hemocyto-genetic form of Kupfer's cells* would give rise to platelets in platelet deficiency.

DOMINICI and FLÖSSNER believed that platelets originate from *lymphocytes*. JORDAN & SPEIDEL also attributed the origin of platelets to lymphoid cells in the frog and salamanders. LEVI regarded *monocytes in the spleen and lymph nodes* as mother cells of platelets. Already MOSEN considered that platelets originate, principally, from *endothelial cells*. This opinion was later

supported by PATELLA and AYNAUD. MARTINO attributed the origin of platelets to *reticular cells*, whereas MARICONDA and CIOTOLA regarded *histiocytes* as mother cells.

BLACHER and LIVADAS claim to have observed *quite new mother cells* of platelets. The former considered that his thromboplast was an atypical bone-marrow cell. The latter observed his thrombocytoblast in rabbit's blood. These cells would not be identical with the megakaryocyte.

Finally, PORTIER brought forth the curious hypothesis that platelets were some kind of *microbes* («Les symbiotes»).

For the sake of completion it should be mentioned that several authors (STÜBEL, EREDE, SCHILSKY, HUMMEL, PETRI, BARTA, SEGERDAHL) who made the origin of platelets the object of their own investigations, were unable to form a definite opinion on the subject.

## 8. Discussion on the bibliography of the origin of blood platelets.

As seen in the foregoing, the question of the origin of platelets has given rise to an extensive literature. Attempts have been made to attribute the origin of platelets to almost every possible cell in the blood and the blood-producing organs. Unfortunately, many works are incomplete and the full value of them can therefore not be assessed.

It is, however, quite obvious that the majority of hematologists favour WRIGHT's theory. It is also obvious that many of WRIGHT's followers regard his theory as the most plausible although as yet not finally corroborated. The Wright pictures, KAZNELSON's clinical evidence, the agglutinability common to megakaryocytes and platelets, and the whole mosaic of other evidence, render the accuracy of the theory extremely probable. The fact remains, however, that, altogether, the evidence is of a more or less indirect nature. In order to provide the decisive evidence for WRIGHT's theory, the formation of platelets from megakaryocytes must be observed directly. FIESCHI & ASTALDI tried without success to observe it in bone-marrow cultures.



Of all the other theories, SCHILLING's theory and its modifications is the only one which, hitherto, has been regarded as a possible alternative. This theory has received much opposition, but rests, nevertheless, on many acceptable arguments. Quite objectively, however, it must be admitted that the evidence is more indirect than in the case of WRIGHT's theory. The idea that the used-up nucleus of the normoblast would have a new and important mission to fulfil also seems to conflict with elementary biologic laws. Nevertheless, decisive evidence against SCHILLING's theory is still lacking.

The rest of the theories seem no longer to be of any topical interest. The conception of platelets as cells has, in most quarters, been abandoned, and it has been found that the structure of platelets argues against formation from mature red blood corpuscles or through precipitation. Nor are the leukocytes mentioned any longer as possible mother cells of platelets. The rest of the hypotheses are interesting only as curiosities.

The term pseudoplatelets has been introduced. With this is understood platelet-like bodies which lack the properties typical of true platelets. DOWNEY and BIRCH asserted on good grounds that all platelet-like bodies which do not originate from the megakaryocytes, are pseudoplatelets.

The prospect of future investigations seems principally uncomplicated. It is a matter of following the whole process of the formation of platelets. On the other hand, it is matter of finding decisive evidence against SCHILLING's theory, in other words, of giving a different explanation of the fate of the nucleus of the normoblast. The fact that such a great number of authors have attacked the problem indicates that it is not easily solved. It seems to me, however, that renewed studies of platelets and bone-marrow cells on the basis of cell structures not considered hitherto, would be worth while in the hope of bringing the investigations on the origin of platelets one step closer to the solution of the problem.

### III. The author's investigations.

The observations made in the present investigation are closely associated with I. WALLGREN's findings with regard to the microscopic structure of the living substance. In native preparations of blood which he examined in transmitted light, WALLGREN observed that the cytoplasm of eosinophile and neutrophile granulocytes contained an abundance of small, pale refractive drops. The drops did not stain with vital stains and were always observed in the vicinity of other, vital stainable drops which also in an unstained condition appeared dark and were less refractive. SCHULTZE, already in 1865, made similar observations in living leukocytes, although his findings have been forgotten. WALLGREN, furthermore, found that the two kinds of drops were recognizable in most blood and bone-marrow cells in native preparations, and that they were observable also in fixed materials in different kinds of tissue cells.

The white blood corpuscles have earlier been studied closely in native preparations by means of dark-ground illumination chiefly by SCHILLING (368), V. JAGIĆ and A. WALLGREN. The latter in particular devoted much time and interest to these investigations. In the cytoplasm of the leukocytes examined in dark-ground illumination, these investigators observed numerous luminous granules which they assumed to be identical with those seen as stained granules in transmitted light. I. WALLGREN, however, found that the refractive drops described by SCHULTZE and him, shine also in dark-ground illumination; whereas the earlier known stainable drops are optically void. Thus, the latter drops appear black and are therefore less easy to detect. They have also been overlooked entirely in dark-ground illumination by all previous investigators.

On the basis of his observations I. WALLGREN (472) describes the microscopic structure of the cytoplasm as follows.

»In the cytoplasma there are two different kinds of drops. The dark drops are less refractive than the surrounding medium. Due to optical reasons they seem to be black. They refract the light in the same way as concave lenses and do not draw the light to a spot when the tube is elevated. Adjoining them we see the pale drops which are more refractive than the surrounding medium. They are similar to convex lenses and appear distinctly when the tube is elevated because the light is collected to a white spot, a picture of the diaphragm-opening above the drop. If we hold a characteristic object, for instance a pin head, before the diaphragm aperture a corresponding shadow appears above each of the pale drops. This is an optical proof of there actually being pale drops. The two kinds of drops are in motion in the living substance, each one in its own characteristic way. The pale drops often flow together and the dark drops communicate by means of threadlike bridges. Thus there are two different kinds of drop systems which are intimately mixed.»

I. WALLGREN also observed that the dark and the pale drops in many cases change places in relation to each other, in those portions of the cytoplasm where the drops are abundant. In some parts of the cell body, however, where the drops have been isolated by the surrounding structureless hyaloplasm, the dark and the pale drops cling together stubbornly and stick to each other also after they have been extruded from the cell. WALLGREN therefore assumed that the two drop systems are carriers of different electric charges.

I. WALLGREN noted that also the cell nucleus and the Golgi apparatus were to a large extent built up of the double drop system.

During my investigations I have, naturally, tried to form an opinion on I. WALLGREN's observations. All the observations which have been cited above as evidence for WALLGREN's views, have been corroborated without difficulty. It has been particularly interesting to follow the movements of the granular substance. It seems to me that the above-mentioned observations cannot be explained unless the cell were built up of two different kinds of granules or drops. In the field of histology, I. WALLGREN appears to have made observations which cannot be

neglected without weighty reasons, especially as they have lead him to discoveries of considerable importance in cytologic and hematologic investigations.

The structure of platelets and alterations in platelets in native preparations have been the subject of study on the basis of examinations in dark-ground illumination. FONIO & SCHWENDENER, in a recent monograph, have discussed the question in detail. These investigations, however, have not brought the problem of the origin of platelets nearer its solution. So far as I can find, the megakaryocytes have been studied in dark-ground illumination only by TORRIOLI and his collaborators, who, however, appear to have made no particularly important contributions to the question. In the present investigation the first task was to compare the structure of platelets and megakaryocytes in dark-ground illumination. Further, I compared the development of these elements in native preparations, observing the megakaryocytes in the hope of being able to witness the process of platelet formation from these cells. At the same time a comparison was made between the structure of platelets and that of all other normal constituents of the blood in order to find, as far as possible, evidence for and against the different theories on the origin of platelets.

The fact that the nucleus of the normoblast besides the cytoplasm of the megakaryocytes has been regarded as a possible mother substance of platelets, gave, naturally, rise to the hope of finding a decisive explanation of the fate of this cell nucleus. I. WALLGREN's (472) investigations have also presented an opportunity in this respect, as this author has demonstrated that the development of the normoblast can be followed in bone-marrow preparations. Finally, SCHILLING's and ØRSKOV's observations, which are difficult to explain, have lead to control investigations.

## 1. Material and technique.

The plan of work outlined above required a varied collection of examination material. The structure and development of platelets were first studied in peripheral blood. Megakaryo-

cytes and normoblasts were examined in bone-marrow preparations obtained by sternal puncture (according to Arinkin). The sternal punctates were obtained from healthy persons as well as from hospital patients. With regard to the hospital patients, no case was included where the megakaryocytes could be expected to have undergone previous pathologic changes, qualitatively or quantitatively. In most cases gastric or duodenal ulcer was diagnosed. No qualitative or quantitative pathologic changes in the megakaryocytes were observed in any of the sternal punctates. Sternal puncture was performed on 5 healthy persons and 25 hospital patients.

The preparations were produced in the following manner. A small drop of blood or material obtained by sternal puncture was procured with the slightest possible trauma. The drop was allowed to spread and form the thinnest possible layer between the object glass and the cover glass, whereupon the cover glass was surrounded with vaseline. In the examinations of the bone-marrow ordinary smears were produced simultaneously, which were stained according to May-Grünwald-Giemsa. A total of 150 native preparations of bone-marrow and a slightly smaller number of smears were examined. Vital staining with brilliant-cresyl-blue (Hollborn) was done in a few cases.

On recapitulating SCHILLING's and ØRSKOV's experiments the instructions given by these authors were followed as strictly as possible. The arrangements for the experiments will be described in detail later.

In the examinations in dark-ground illumination Zeiss' cardioid condenser was used with Leitz' Monla lamp as the source of light. The optical system consisted as a rule of Zeiss' apochromatic objective 120 W,  $\text{nA } 0.85 \times \text{K } 10, 15, \text{ or } 20$ . When living cells are injured by light, filter OG<sub>2</sub> was used.

In parallel studies in transmitted light, the light was obtained through Zeiss' pancartic condenser, whereas the optic system consisted of Zeiss' apochromatic objective H 1 90  $\times \text{K } 10, 15, \text{ or } 20$ .

The photomicrograms were taken with a Zeiss Ikon Contax on Agfa Isopan F and Ansco Color 235 film. On photographing

stained cells on dark-white film a dark green filter was inserted into the cone of light.

Another noteworthy fact is that the examinations in dark-ground illumination require object glasses which are thinner than 1.2—1.3 mm. The glass must be of a thickness which allows the condenser to gather the light in a point at the level of the object studied, at least when the condenser is placed at maximal height.

## 2. Observations on platelets.

### *a. The structure of platelets.*

The structure of platelets, which appears in stained (e.g. May-Grünwald-Giemsa) smears in transmitted light, is well known. A granulated portion, so-called granulomere, may be distinguished from a structurless portion, so-called hyalomere. On the examination of the same preparation in dark-ground illumination a group of luminous granules may be seen at the site of the granulomere, whereas the hyalomere appears to be optically void (see figs. 11 and 12). In these stained preparations the granules have a greenish hue. A number of authors regard the granulomere as a nucleus. This view was supported most recently by FONIO (142).

In his latest communication I. WALLGREN (472) demonstrates that the granulomere of platelets is built up of the double granular system described above (see pages 30—31), and that dark and pale drops intimately mixed are to be seen in this structure.

WALLGREN also points out the remarkable fact that the granulomere covers a larger area in dark-ground illumination than in transmitted light. This seems to indicate that some dark drops, at least in the peripheral parts of the granulomere, do not stain.

The investigations described in the following bear out WALLGREN's observations. A few more details are perhaps worth mentioning. The granulomere of platelets, observed in dark-ground illumination, appears to contain different kinds of

luminous granules, larger and more luminous, smaller and less luminous forms of granules. Already STÜBEL made similar observations. SABIN also describes two kinds of granules, but it is difficult to decide whether he observed the same phenomenon. The size of these granules varies within limits which are the same for all platelets. As will be seen in the following, it has been possible for me, already on the basis of this fact, to establish significant differences between platelets and most other cell structures. I have not been able to judge the size of the dark granules with sufficient accuracy. No sign of a membrane of a nucleus has been found. In most cases the arrangement of the granules seemed to indicate that the granulomere is not a nucleus. The shape of the granulomere varied considerably in many cases. The structure of platelets was of the same appearance in stained and fixed as well as in native preparations on examination in dark-ground illumination. The double granular system, that is, intimately mixed dark and pale drops, appeared to be distinctly observable in both preparations.

WOLPERS & RUSKA examined the platelet structure in the electron microscope. They found that a larger number of granules are observed with this method than with the generally accepted technique. Otherwise, however, no important results in this field seem to have been obtained by their method.

#### *b. Changes in platelets in native preparations.*

The question whether platelets should be regarded as living elements is intimately associated with the changes in the shape of platelets and their finer structures. As may be seen from the historical survey, many authors consider that platelets are living elements, indeed, complete cells. A. WALLGREN has defined the movements in the pale granular system of the cell, which are typical of the living cell substance, and I. WALLGREN has described the movements characteristic of the dark granular system. He has never been able to observe movements in the drop system in platelets.

The changes in shape which platelets undergo in native preparations, have since long been the object of general interest, al-

though, periodically, many discoveries have rested forgotten. Undoubtedly, already HAYEM (182), in 1879, observed the formation of blisters, typical of platelets. It has later been described by PUCHBERGER, ROSIN & BIBERGEIL, NATTAN-LARRIER, STÜBEL, GÁSPAR, TAIT & BURKE, WATSON, BOSCH, FONIO, WOLPERS & RUSKA, SONDER, SCHWENDENER and I. WALLGREN (472). It is obvious that several of the said authors have made their observations without being aware of the works of their predecessors. WOLPERS & RUSKA regarded the blisters as vacuoles. Also VON NEERGAARD appears to have observed them but seems to have mistaken them for parasites. BOSCH considered that cooling, contact with glass, etc., were the causes of these phenomena.

The earlier observations were made in transmitted light. The introduction of examinations in dark-ground illumination has greatly facilitated the observations. STÜBEL was the first to examine platelets in this kind of illumination. FONIO & SCHWENDENER's monograph contains, largely, all that we know at present about the changes in platelets in native preparations.

FONIO & SCHWENDENER worked partly with whole blood, partly with blood free from leukocytes and erythrocytes. A 14 % solution of magnesium sulfate was added to the blood in order to prevent the agglutination of platelets. Immediately after the preparations had been produced, they lay like discs with sharp contours. Very soon short, bristle-like protrusions of protoplasm («pseudopods») appeared, which were subsequently elongated and assumed double contours. The bases of the protrusions became gradually broader and the protrusions themselves disappeared. Platelets now formed a granulated substance surrounded by a structureless seam. According to FONIO & SCHWENDENER a »Ruheform» had developed from a »Reizform» (with »pseudopods»). Bag-shaped pseudopod-like protrusions were then observed, which finally formed round blisters with a few granules in Brownian motion. For the sake of clarity it should be mentioned that FONIO & SCHWENDENER thus assumed that there were two kinds of pseudopods, the threadlike kind characteristic of the »Reizform», and the broad kind formed from the »Ruheform». According to them the said blisters consisted of platelet protoplasm (they were inclined to regard platelets as complete



cells) and may also develop directly without appearing first as pseudopod-like protrusions. The blisters could then be liberated from the remaining granulated substance. I. WALLGREN (472), recently, described similar changes in platelets without being aware of the above-mentioned investigations.

In my observations I worked principally with untreated blood. The preparations were placed as quickly as possible on the heating table of the microscope, which had a temperature of 37° C. The following observations were recorded:

When the preparation was ready for examination (about 1 minute after the blood had been procured) all the platelets appeared rounded off without either narrow or broad pseudopods. A certain degree of agglutination of platelets was observed in some preparations. In all the preparations, however, most of the platelets were free.

After approximately 2—3 minutes structureless seams or rims gradually spreading out, appeared about the platelets. Many platelets were surrounded completely by these rims, but just as often they were restricted to a part of the periphery. A small number of platelets never showed any rims, nor did they undergo any other changes. Whether these belong to the true platelets cannot, naturally, be decided with certainty, but the structure differed in no way from that of the others. In these preparations I was only exceptionally able to observe bodies resembling FONIO & SCHWENDENER's threadlike »pseudopods».

The rims grew slowly and irregularly and many of them gave the impression of pseudopods. They were seen frequently to shrink temporarily, which strengthened the impression. When these »pseudopods» had reached a considerable size ( $\frac{1}{2}$ —1 platelet diameter) one or a few granules were in many cases liberated from the granulated substance, to be shifted slowly toward the periphery of the »pseudopod», where they to begin with lay more or less immobile. This phenomenon, however, was observed only in a small number of platelets.

After 10—15 minutes (calculated from the time of the blood-letting), the first blisters began to appear. They arose visibly from the pseudopod-like bodies, but the development of blisters without a pseudopod-like phase was also observed. SCHWEN-

DENER has drawn attention to this fact. I find it difficult, however, to rule out the possibility that a pseudopod stage has been overlooked.

When the growth of the blister was completed, free granules present within the blister began to show Brownian movements. Some Brownian-moving granules appeared the moment the blister developed. FONIO & SCHWENDENER found 2—4 leaping granules in each blister. This is a usual number but many more granules were observed fairly frequently. I have on different occasions seen blisters with 20—30 granules. Nor was it particularly unusual to observe blisters in which not a single granule could be recognized, despite minute examination.

FONIO & SCHWENDENER, in their monograph, designate these blisters as »Protoplasmaringe». I found, however, that many platelets after the completion of the formation of the blisters had grown into many times the original size. Platelets may possibly contribute substance, but it seemed to me that they during the formation of rims and blisters definitely absorbed material from the surroundings. This opinion has been advanced earlier by WOLPERS & RUSKA. I. WALLGREN (472) has also expressed the same view. He points out the change in viscosity that apparently takes place during the formation of blisters and which is manifested distinctly in the Brownian movements of the granules.

There seems to be some haziness as to the nature of the above-described changes in the platelets also in another respect. Should the growth of pseudopods and blisters be regarded as manifestations of life? According to SCHWENDENER they are phenomena of decomposition.

In order to throw some light on this question, certain experiments were carried out in which poisons were added and the preparations frozen. Among the poisons used in the experiments was magnesium sulfate (14 %), that is, a similar fluid to that used by FONIO & SCHWENDENER in their investigations. This fluid, already in a relatively low concentration, destroys all white blood corpuscles. The amount of  $\text{MgSO}_4$  per preparation used in the present experiments appears to be considerably smaller than that used by the said authors. No manifestations of

life, however, were observed in any of the white blood corpuscles. The changes in the platelets themselves in these preparations were identical with those described by FONIO & SCHWENDENER. The thin »pseudopods» that they had observed, were also seen here. Broad pseudopods and blisters were present in the usual number.

The same result was obtained on vital staining with brilliant-cresyl-blue. In weak concentrations this staining medium did not affect the manifestations of life in the white blood corpuscles, whereas, for instance, in the concentration used ordinarily in staining of reticulocytes it prevented every sign of life. The changes in the platelets, the formation of pseudopods and blisters, however, proceeded in the normal manner also in a high concentration.

In order to be able to rule out every possibility that the changes in the platelets were manifestations of life, preparations to which potassium cyanide was added, were produced. Equal volumes of blood and 3 % KCN were mixed. When the preparation was placed on the heating table of the microscope, numerous threadlike protrusions were seen on the platelets. They resembled exactly FONIO & SCHWENDENER's »Reizform». The broad pseudopods appeared after a few minutes, whereupon they gradually and somewhat slower perhaps than without the addition of poison, grew into typical blisters.

The experiments with freezing were less well suited to illustrate this question. White blood corpuscles also retained their ability to show movements of life over a period of freezing at 0°—about -5° C. On the other hand, very low temperatures caused changes in the preparations which were so marked as to render the observations difficult. It should be pointed out, however, that freezing for 1 hour at -5° C. did not affect the changes in the platelets after the preparation had been allowed to melt again.

Finally, it should be mentioned that a typical formation of blisters about the platelets was observed also with the fixation technique used by ØRSKOV in his experiments (see page 65).

On studying the development in native preparations some of the granules that had not passed into the blisters could be

seen, now and then, to leave the platelet body, suddenly. When they had entered the blood-plasma these granules, or rather pairs of granules consisting of a dark and a pale drop, began to show Brownian movements, whereupon it became impossible to distinguish them from ordinary hemokonia. It seems probable to me that the increase in hemokonia, which was always noticed in native preparations, was due, partly, to the passing of the platelet granules into the blood-plasma. It was, however, certain that the platelets alone did not cause this increase. Differences in the size of the hemokonia and the fact that the formation of hemokonia from the most widely different blood cells could be observed showed that the hemokonia at least in native preparations were of varied origin. It seems to me, however, difficult to determine to what extent hemokonia, on the whole, are present in circulating blood. Certainly, these investigations do not justify any definite conclusions as to the possible formation of hemokonia *in vivo*. Even the smallest trauma caused formation of hemokonia in these native preparations and traumas may hardly be avoided altogether in the production of the preparations. It will be necessary to revert to this question later in this work.

A description of the changes in the platelets in native preparations would be incomplete without a few words about the relation of platelets to the formation of fibrin. The fibrin precipitation is more marked in some preparations than in others. As a general rule, however, it may be established that the fibrin arranges itself in a typical manner about the platelets and clumps of platelets. The fibrin does not precipitate about any other normal constituents of the peripheral blood.

### 3. Pseudoplatelets.

Besides the true platelets which were distinguished by the above-described characteristics, practically all native preparations contained small granulated elements which could readily be mistaken for platelets. On close examination, however, the granules of these elements were found to be slightly larger than

those of true platelets. As distinct from platelets, all the granules in one of these pseudoplatelets were of the same size. There was no observable formation of »pseudopods» or blisters. The structure was identical with that of the neutrophile granulocytes and they should in all probability be regarded as fragments of these. This seemed highly probable especially as, now and then, these elements were seen to be extruded from the leukocytes. Light pressure exerted on the cover glass yielded large quantities of these pseudoplatelets.

Not only neutrophile granulocytes were found to give rise to pseudoplatelets. Small platelet-like cell fragments were sometimes observed, made up of the coarse granules which are typical of the eosinophile granulocyte.

In those cases where the formation of pseudoplatelets was observed directly, the granules involved never showed any signs of life. It is natural to assume that these pseudoplatelets are artefacts which have arisen in connection with the trauma caused by the bloodletting, especially as they are obtained so easily on extra traumas. I am not able to judge whether pseudoplatelets may be present in circulating blood.

An unusually large number of pseudoplatelets was sometimes observed in the blood of severely affected patients. On the basis of my limited material I have not been able to ascertain whether the phenomenon is associated with certain diseases. BIRCH reports an increase in the number of pseudoplatelets in cases of chronic splenomyelogenous leukemia. It is, naturally, difficult to decide whether such cases are conditioned by a reduced resistance to traumas in the leukocytes of these patients or whether the pseudoplatelets for some reason were formed already *in vivo*.

#### 4. Observations on megakaryocytes.

The nature and the origin of the megakaryocytes has been the subject of much discussion. This problem is largely outside the scope of the present communication. It may be mentioned, however, that the majority of investigators nowadays consider

that the megakaryocytes originate either directly from the myeloblast or from a mother cell common to the myeloblast and the megakaryocyte. Much evidence of the relationship between the megakaryocytes and the myeloid cells has been adduced and this relationship seems now to be placed beyond all doubt. Also endothelial cells, however, have been mentioned as mother cells of the megakaryocytes.

The observations which will be described in the following were made on stained smears (May-Grünwald-Giemsa) as well as native preparations. As regards the stained smears the observations have not been recorded in detail, as this procedure would be of no significance. Detailed records have been made of the examinations of native preparations. By chance, exactly 200 megakaryocytes in native preparations have been recorded. As already mentioned, these are distributed among 150 preparations from 30 persons.

#### *a. The structure of the megakaryocyte.*

It is a known fact that the mature megakaryocyte in smears of bone-marrow stained according to accepted methods has a finely granulated structure. The size of the granules varies in these stained preparations. The variations in the size of the granules caused at first certain objections to WRIGHT's theory. It was difficult to find megakaryocytes with granules of the same size as in platelets. NAEGELI (298), however, demonstrated quite clearly that all transitional stages, from seemingly non-granulated cytoplasm to cytoplasm with granules of the same size as in platelets, may be observed in the megakaryocytes.

So far as I can find, megakaryocytes have been studied in dark-ground illumination only by TORRIOLI and his collaborators. They worked with native preparations. Although they supported WRIGHT's theory, their methods, which had not been used before in examinations of megakaryocytes, do not appear to have given any important contributions to the question of the origin of platelets.

The megakaryocyte stained according to May-Grünwald-Giemsa presented in dark-ground illumination a beautiful

picture (see fig. 3). The entire protoplasm appeared to be composed of granules. Luminous granular forms of various size were seen. Dark granules appeared between the luminous granules. Thus, also the cytoplasm of the megakaryocyte seemed to be built up according to the principles devised by I. WALLGREN. The whole cell gave the impression of being green. It was impossible to judge with certainty whether the luminous granules had stained or whether the colour came from the hyaloplasm. The dark drops had obviously stained deeply.

The nucleus of the megakaryocyte was, principally, built up in the same way. The granules, however, appeared less distinctly. The nuclear structure seemed to become more indistinct in the more mature cells. It was often difficult to observe the structure in those megakaryocytes which appeared to give rise to platelets.

The examination of megakaryocytes in native preparations in dark-ground illumination revealed, principally, nothing new. It was easy to find the cell also in unstained preparations on account of its size and characteristic structure. Because of its relatively fine granules it was difficult to study the megakaryocyte in transmitted light in native preparations, but also this examination seemed to reveal two different granular systems, dark and pale drops.

A comparison of the structure of platelets and megakaryocytes in dark-ground illumination showed that they were identical (see figs. 8 and 9). The structures were so much alike that it was impossible to distinguish a megakaryocyte from a clump of platelets, unless a cell nucleus was discovered (which may be difficult, sometimes, in native preparations). In some cases the megakaryocyte was more sharply outlined than the clump of platelets, but this was fairly rare. In the present work only those cells in which a nucleus could be recognized definitely, were recorded as megakaryocytes.

The typical pictures which according to WRIGHT and many other authors after him, represent phases in the formation of platelets, have also been made the object of study in the present investigation. In stained smears of bone-marrow it was not very difficult to find megakaryocytes the protoplasm of which

appeared to be divided into clumps of granules the size of platelets (see fig. 4). Nor was it unusual to find megakaryocytes where the cytoplasm at the periphery seemed to break up into platelets (see fig. 6). WRIGHT's interpretation of such findings is extremely obvious.

Studied in dark-ground illumination, however, such cells presented a surprising picture. A completely homogeneous distribution of dark and pale drops could be observed everywhere (see figs. 5 and 7). There was not the slightest sign of a platelet-like accumulation of granules. The only explanation of this finding seems to be that all dark drops do not stain identically. I am not able to judge whether dark drops with different stainability are present primarily, or whether some of these drops lose their stainability at the onset of the formation of platelets.

The finding harmonizes with I. WALLGREN's observation that the granulomere of platelets in dark-ground illumination is markedly larger than the stained granulomere in transmitted light (see figs. 4—7).

On studying native preparations I have observed on one occasion an unstained megakaryocyte in dark-ground illumination, the cytoplasm of which was divided into platelet-like clumps of granules. As the cell after staining could not be studied in transmitted light, it is extremely difficult to judge the significance of this observation.

#### *b. Changes observed in the megakaryocyte in native preparations.*

The first task was to study the possible signs of life that may be observed in the structure of the cytoplasm of the megakaryocytes. TORRIOLI was not able to observe movements in the megakaryocytes in his native preparations, but he referred, probably, merely to shifting and change in the shape of the cell. It should be pointed out at once that the great majority of megakaryocytes show no shifting of the granules of the kind which according to A. and I. WALLGREN is typical of life. I seemed to notice such movements in only 4 cells (2 per cent). They were, however, isolated and extremely slow. These sporadi



observations do not justify the ruling out of errors or other kinds of displacement.

Why do the megakaryocytes, generally, show no typical manifestations of life? There may be different explanations. The trauma caused to this giant cell at the sternal puncture and in the production of the preparation is naturally far greater than that affecting the smaller cells. Should the general conception that the megakaryocytes are situated extravasally be correct, the trauma may be still greater. On the whole, we know nothing about the possibilities of the megakaryocytes to live outside their natural environment.

On the other hand, the considerable resistance to various traumas shown by the megakaryocytes must be emphasized. FIESCHI & ASTALDI point out their power of resistance in bone-marrow cultures. In my investigations they have shown a greater resistance to traumas than any other cells in the native preparations and have disintegrated only on very heavy pressure on the cover glass. Also here the possibility of a form of life which is not manifested in movements in the cytoplasm must be considered.

Scant attention has hitherto been paid to the changes in the megakaryocytes in native preparations. So far as I can find, WRIGHT, 1906, in his important work, is the only author who reports that the hyaline marginal zone in giant cells performs protoplasmatic movements similar to those observed at the periphery of platelets.

Unfortunately, it was possible for me only in a few instances to start the observations immediately after the production of the preparations. On account of the long distance between the laboratory and the place where most of the sternal punctures were made, the greater part of the observations could not be started until 20 minutes—1 hour after the specimen had been procured. The interpretation of the results, however, does not seem to have suffered in any noteworthy degree through this disadvantage.

In the few cases where the examinations could be started some minute after the specimen had been procured, it was found that the behaviour of the megakaryocytes had much in common

with that of platelets (see fig. 10). After a few minutes of observation pseudopod-like rims began to protrude from most megakaryocytes. The rims, principally, resembled completely the corresponding pseudopods of platelets. After 15—30 minutes, blisters which could not be distinguished from the platelet blisters, developed from these rims.

Many of the blisters on the megakaryocytes contained granules showing Brownian molecular movement. Blisters free from granules, however, were perhaps seen more frequently in the megakaryocytes than in platelets. A remarkable fact was that the blisters did not develop only along the periphery of the megakaryocyte. In many cases they were observed above or underneath the cell, that is, projected towards the central portions of the cell. In exceptional cases the blisters were so numerous that they, side by side, covered the entire cell.

It was not unusual that no formation of blisters about a megakaryocyte was noticed. The observations were often difficult, for instance with fairly thick preparations. In the present work I have recorded the formation of blisters only in absolutely certain cases. Thus, it was found that 135 megakaryocytes, or 67.5 per cent, showed definite blisters. Profuse formation was recorded in 54 cases, moderate in 42 and sparse in 39 cases. As object of comparison was used the average number of blisters in a clump of platelets the size of the megakaryocyte in question. These figures may be regarded as absolute minimum values.

The question may naturally arise, whether variations typical of some pathologic conditions may be observed in the intensity of the formation of blisters. My material is obviously too small to permit of any definite conclusions. It seems, however, highly improbable, judging from the very wide range of variations which I noticed in the bone-marrow preparations from the thoroughly healthy objects of examination.

A different type of blisters was observed in a few cases. There, a considerable portion of the cytoplasm of the megakaryocyte broke up into round or oval bodies up to the size of leukocytes. These were completely filled with granules showing Brownian molecular movement. Parallel with this phenomenon

the usual formation of blisters was observed about the same cell. In some cells it was also possible to follow, actually, all the transitional stages from the small to the large blisters. These large blisters were observed in 13 cases (6.5 per cent).

A remarkable finding was the accumulation of fibrin appearing about some megakaryocytes. With the exception of platelets, no accumulation of fibrin whatsoever could be demonstrated about any other elements in the native preparation of the bone-marrow. Although far from all megakaryocytes presented this phenomenon, it was, nevertheless, observed about 40 cells (20 per cent). A profuse amount of fibrin was recorded in 9 cases, moderate in 20 and sparse in 11 cases. Also in these cases average clumps of platelets were used as object of comparison. Although the phenomenon was not constant it seems to me to have been observed in a sufficient number of cases to justify the ruling out of every suggestion of chance. The above-mentioned figures may be regarded as minimum values.

*c. The pinching off of cytoplasm from the megakaryocytes.*

In the present examinations of native preparations my interest was largely concentrated on the possibilities of following directly the formation of platelets from megakaryocytes. I was unsuccessful in this respect. This disheartening fact has many possible explanations. The most obvious reason is that the megakaryocytes were injured when taken from their natural environment. The absence of manifestations of life, mentioned in the foregoing, renders this explanation most plausible.

Frequently, however, there were seen in native preparations megakaryocytes which to a larger or smaller extent were surrounded by granulated elements. These were either detached fragments of the cytoplasm of the megakaryocytes or platelets which, on account of the common agglutinability, had collected about the megakaryocytes. Such findings were recorded for 55 megakaryocytes or in 27.5 per cent of the total number. These elements showed all the changes described above as typical of platelets.

All these bodies were fully developed when the microscopic examination started. In no case was the examiner able to witness the formation of these platelet-like constituents of the blood. One observation, however, may deserve to be mentioned.

A megakaryocyte with fairly sparse cytoplasm and without observable formation of blisters was made the object of close study. It showed no signs of life whatsoever. After a while a couple of neutrophile granulocytes approached the large cell. These leukocytes made their way into folds in the cytoplasm and attacked the megakaryocyte vigorously. After a few minutes fragments of cytoplasm were liberated. Most of the fragments remained lying in the vicinity of the cell. Shortly, however, one of the leukocytes left the megakaryocyte. By means of threads it was attached to two fragments of the megakaryocyte cytoplasm which it towed out of sight like an engine with two carriages. Subsequently other leukocytes appeared and all the cytoplasm was liberated from the nucleus of the megakaryocyte. Thus, at the end of the observation the nucleus lay at some distance surrounded by numerous platelet-like pieces of cytoplasm. These did not, however, behave like platelets. They did not form blisters, nor were they surrounded by fibrin. It seems scarcely probable that this process would represent the course of events in the normal formation of platelets. I was not able to make another similar observation.

## 5. Comparison between platelets and megakaryocytes.

The following is a summary of comparative studies of platelets and megakaryocytes, within the framework of the above-described observations.

Structural similarities in fixed and stained preparations studied in transmitted light have been reported earlier in the literature but have also been considered a debatable fact. Certain variations may be demonstrated in transmitted light. When platelets and megakaryocytes are studied in dark-ground illumination, however, they show an identical structure (see fig. 9).

Both have larger and more luminous, smaller and duller granules. In both cases, however, the size of the granules varies within the same limits.

Also as regards the changes in native preparations the two elements show characteristic similarities. The formation of blisters typical of platelets is also observed about the majority of megakaryocytes. Most of the blisters on the megakaryocytes resemble exactly those observed about the platelets, although blisters of a different type may be seen about some megakaryocytes.

As is known, platelets and clumps of platelets are in most cases surrounded by fibrin after having been allowed to stand for a while in native preparations. No general accumulation of fibrin about the megakaryocytes has been demonstrated. 1/5 of the total number of megakaryocytes, however, have been surrounded by accumulated fibrin. In view of this frequency it seems to me that the possibility of coincidence may be ruled out, especially as no formation of fibrin has been observed about other blood and bone-marrow cells.

## 6. Comparison between platelets and other blood and bone-marrow cells.

As has been mentioned, attempts have been made to attribute the origin of platelets to practically all the cells in the blood and bone-marrow. It is impossible, however, in the present communication to compare them in detail with all other cell forms. A review of the history shows on which points a comparison may be of some significance.

The relationship between the *nucleus of the normoblast* and the formation of platelets will be described in detail later. Suffice it to say here that the structure of the nucleus of the more mature normoblast is extremely indistinct and bears no resemblance to the structure typical of platelets.

The mature erythrocyte when examined in dark-ground illumination, is optically void. It shows no sign of granulation. It

seems hard to believe that this structureless blood corpuscle would be capable of producing platelets with their typical granulation. It is more obvious to assume that platelets originate from cells which already in themselves are granulated, especially as all other blood and bone-marrow cells contain granular substance.

The granulation is not the same in the different leukocytes. The *eosinophile granulocytes* have the coarsest granules of the cells in human blood and bone-marrow. These granules are far larger than in platelets. The same applies to the *neutrophile granulocytes*, although to a lower degree. In these cells all the luminous granules appear to be of the same size. The difference between the granules of platelets and those of the neutrophile granulocytes is observed more easily in native preparations than in stained smears. What has been said here about the granules of the different granulocytes applies also to the precursors that may be studied in material obtained by sternal puncture. STÜBEL already in 1914, studied leukocytes and platelets in dark-ground illumination and has drawn attention to the above-described dissimilarities. J. WALLGREN (467), recently, has described the finer structure of the white blood corpuscles, without comparing them with platelets, however.

The *monocytes* are, intentionally, discussed separately in the present communication. I. WALLGREN (467) has pointed out that the luminous granules of these cells are of unequal size. On comparison with the luminous granules of platelets the similarity is striking. There is no demonstrable divergence. Thus, beside the megakaryocyte the monocyte is the only cell in blood and bone-marrow, which shows a similar structure in dark-ground illumination.

The size of the granules in the Golgi apparatus of the *plasma-cells* falls within the limits of the size of the platelet granules, but they are always of equal size in relation to each other. The Golgi apparatus of the *lymphocytes* contains principally luminous granules which are markedly smaller than the granules of the platelets. I. WALLGREN's investigations throw light also on this question.

Is it possible to observe also in other cells phenomena which resemble the formation of blisters typical of platelets? I have never succeeded in this respect. It may be mentioned however, that different white blood corpuscles, in particular, which have been injured by poisons (e.g. KCN) or traumas and show no further signs of life, may exhibit rims and pseudopod-like bodies which resemble principally the typical changes in platelets. No actual blisters are formed. In dark-ground illumination the rims on the leukocytes show coarser outlines than the blisters of the platelets and are always optically void. Thus, no Brownian-moving granules are ever observed in them. This applies also to the monocytes which have been made the object of particularly close study.

Nor has it been possible to observe a definite accumulation of fibrin about any other elements than platelets and megakaryocytes. In the rare cases which seemed to show a suggestion of it the possibility of coincidence could not be ruled out.

## 7. The normoblast; its development into an erythrocyte and possible connection with the formation of platelets.

The fate of the nucleus of the normoblast has also been the subject of much controversy among the hematologists. Is the nucleus extruded or does it dissolve intracellularly? The former view, advanced by RINDELEISCH, attracted for a long time many followers, EHRLICH, among others. In recent years SCHILLING has been one of the few spokesmen for this theory. The majority of present-day investigators appear to favour the conception of intracellular dissolution of the nucleus. The extruded nuclei which may be observed without difficulty in all kinds of preparations are regarded as artefacts [NAEGELI (300), and others]. It should be pointed out, however, that the latter theory is also based on indirect and inconclusive evidence.

Already in the general survey of the history I discussed the theory according to which platelets are derived from the extruded nuclei of the normoblasts or portions thereof. Many investigators have followed this line of thought. Since the appearance

of WRIGHT's theory in 1906, however, SCHILLING has been the chief supporter of the »Plättchenkern» theory. As late as 1943 (382) he was still disinclined to abandon it, as already mentioned, although he seemed then to accept WRIGHT's theory as the most plausible. He insisted, however, on a different and decisive explanation of the fate of the nucleus of the normoblast, before he could abandon his conception of this element as a possible origin of platelets. During the last decade also ØRSKOV, in a series of works, has supported this theory, although in a slightly modified form.

In order to elucidate the question of the origin of platelets from as many aspects as possible, I have compared the structure of the normoblast nucleus and of platelets in dark-ground illumination. Furthermore, in accordance with SCHILLING's demands, I have tried to find an explanation of the fate of the normoblast nucleus, following closely the methods devised by I. WALLGREN and guided by his recent work. Finally, I have applied these methods in the recapitulation of some of the experiments on which SCHILLING's and ØRSKOV's theories are based.

#### *a. The structure of the normoblast nucleus.*

Similarly to the cytoplasm and the nucleus of the white blood corpuscles, the nucleus of the normoblast, according to I. WALLGREN (468), is also built up of dark and pale drops. In dark-ground illumination the granulated structure of the nucleus appears particularly distinctly. The structure is far more distinct in native preparations than in fixed and stained smears. Also in the latter the granules are often clearly distinguishable, but the observations are usually rendered difficult by the marked stainability of the nucleus.

In the less developed normoblast the granules of the nucleus are small and of equal size. As a rule they are slightly smaller than the smallest granules of platelets. UNDRITZ & ROTHLIN, in stained smears, seem to have found that the normoblast nucleus loses its structure as it matures and finally gives the impression of a homogeneous »drop». I. WALLGREN (472) has



demonstrated that the distinct granulation typical of the less mature normoblast, when examined in dark-ground illumination, becomes gradually more indistinct. The visible granules become smaller and fewer. The whole nucleus grows smaller. In the most advanced nuclei it is, on the whole, very difficult to discern any granular structure at all.

During the course of the present investigations it has been possible to confirm altogether WALLGREN's observations. The constant difference between the granules of platelets and those of the normoblast nucleus should be emphasized in particular. If platelets were derived from nuclei of normoblasts, it would be obvious to assume that the most advanced nuclei were the source of their formation. It is, however, exactly between these nuclei and platelets that the structural differences are most marked.

*b. The development of the normoblast and the dissolution of the nucleus.*

So far as I can find in the literature there are no reports of studies of normoblasts in native preparations, which would be of any importance to the argumentation in the present work, prior to I. WALLGREN's latest communication (472). This author, therefore, seems to be the first who has published the results of direct studies of the development of the normoblast, not taking into consideration SCHILLING's (371) observations on the formation of platelets from red blood corpuscles in native preparations.

As already mentioned, I. WALLGREN, in 1947, studied living normoblasts in dark-ground illumination. Besides the nucleus, the normoblasts were found to consist of a structureless cytoplasm, for the most part optically void. In this cytoplasm, however, was seen, intimately related to the nucleus, an organ which was changeable as to shape and position and built up of the typical double drop system. This organ has altogether the character of a Golgi apparatus (I. WALLGREN) and will in the following be given this designation.

In a considerable number of preparations I. WALLGREN was able to observe directly the above-described changes in the structure of the normoblast nucleus. The development which was studied chiefly in primarily fairly small nuclei, took place gradually during the course of several hours. The Golgi apparatus changed shape perpetually and the drops were in constant motion in the manner typical of the living cell. Towards the end of this development, that is, when the nucleus was nearly structureless, I. WALLGREN was able to observe in many cases that the nucleus was dislocated out of the cell body, seemingly adhering to the Golgi apparatus. This phenomenon has earlier been described in a similar manner by VALENTINE. After a while it was pulled back into the cell body. Despite a large series of observations, I. WALLGREN was never able to notice that the nucleus was extruded.

In most instances the development stopped at a stage where he observed a small, shrivelled and optically void nucleus. The movements of the Golgi apparatus ceased gradually; the cell seemed to die. It was a long time before I. WALLGREN was able to witness the disappearance of the nucleus.

The origin of the substantia granulo-filamentosa of the reticulocytes has hitherto been unknown. As it is of no immediate significance in the present communication I do not intend to discuss this problem. It should be mentioned, however, that the cell nucleus (NAEGELI, and others) as well as the cytoplasm (FERRATA, SEYFARTH, and others) of the normoblasts has been designated as possible mother substance but no decisive solution has been reached.

In a case of pernicious anemia treated with liver up to the peak of the reticulocyte crisis thus produced, I. WALLGREN was finally able to witness the intracellular dissolution of the nucleus. Liver extract (campolon) had then also been added to the preparation. He observed the gradual dissolution of the nucleus in three normoblasts. The Golgi apparatus remained at first unaffected. The cells had now the appearance, in all respects, of typical reticulocytes. WALLGREN therefore considered that the substantia granulo-filamentosa of the reticulocytes coincided with the Golgi apparatus of the intact normoblast. DAWSON and

DE ROO & UFFORD had earlier made a similar assumption. Single granules were gradually extruded into the blood-plasma. Thus, the reticulocytes became mature erythrocytes.

This observation is probably unique in the literature. Was this an accidental finding? Was this observation conditioned by the patient's illness, the addition of campolon to the preparation, or perhaps by some other non-physiologic circumstances? In other words, should it be inferred that I. WALLGREN was able to follow the normal mode of disappearance of the normoblast nucleus? In order to answer these questions, if possible, I have carried out further observations, applying the methods used by I. WALLGREN and guided by his findings.

In view of the fact that many of I. WALLGREN's observations produced no results, it was decided that no normal cases were to be studied in this connection but only those which may be expected to show an increased blood regeneration. Cases of pernicious anemia in remission seemed to be particularly suitable for the purpose. In this disease the blood formation would reasonably be expected to differ from the normal only as regards the rate. The following 4 cases were studied.

*Case I.* Hemolytic jaundice was diagnosed clinically. Reticulocytes 23.2 per cent. Abundant normoblasts in material obtained by sternal puncture. The preparations were satisfactory. Most of the normoblasts showed distinct signs of life, that is, changes in the shape and position of the Golgi apparatus, and typical shifting of the pale drops. No addition was made to the preparations.

During the observation period, which extended to 24 hours, 7 normoblasts were observed, the majority with indistinct nuclei. During the course of the 24 hours the granulated structure of all the nuclei became gradually more indistinct. In 4 normoblasts the nucleus was dislocated out of the cell and, after a while, drawn back again into the cell body. At the end of the observation period none of the cells showed any signs of life. All the nuclei were still discernible.

*Case II.* Pernicious anemia treated with liver. Reticulocytes 29.2 per cent. Abundant normoblasts in material obtained by sternal puncture. As the previous experiment produced no result, folic acid (approximately 1/10 of the blood volume) was this time added to the preparation. The majority of normoblasts showed distinct signs of life.

Also in this case the observation period was 24 hours. Four normoblasts were observed and depicted. It was found immediately that the movements in the Golgi apparatus were very vigorous. The whole Golgi apparatus changed shape and position perpetually. The nucleus shifted about in the cell. After 1 ½ hour of observation the nucleus of one of the normoblasts was dislocated out, and after another hour a second nucleus. Both were drawn back into the cell body within 10 minutes. After 5 hours' observation the two nuclei were practically structureless and scarcely discernible. The first nucleus to be pushed out became again more distinct after ½ hour. The other cell remained largely unchanged. 11 hours from the start, when the observations were suspended for the night, the nucleus of this cell was not distinctly discernible.

On the following morning, 20 hours after the first observation, it was found that the latter cell had been transformed into an altogether typical reticulocyte (see fig. 1, I). Not the slightest suggestion of a nucleus could be seen, nor was an extruded nucleus found in the surroundings. A considerable number of the granules of the Golgi apparatus were retained but they showed no manifestations of life. These granules were liberated one by one and disappeared from the field of vision in the blood-plasma as Brownian-moving hemokonia. At the end of the observation period only about ¼ of the granular substance of the reticulocyte remained. No signs of life was observed in any cell.

The remaining three normoblasts retained their nuclei. At the periphery of the area under observation were seen two more normoblasts which were not depicted. They were, however, observed incidentally now and then. It was noticed that one of them during the night had been transformed into a reticulocyte. Nor in this case was an extruded nucleus seen in the surroundings. It should be pointed out in particular that no flow of plasma was observed in any of these preparations. It seems therefore almost improbable that an extruded normoblast nucleus could have been pushed too far away from the cell body.

Thus, in this case 2 out of 6 normoblasts kept under observation had lost their nucleus in 24 hours. This had obviously occurred through intracellular dissolution.

*Case III.* Hypochromemia was diagnosed. Reticulocytes 2.1 per cent. Abundant normoblasts in the sternal punctate. As in the previous case, folic acid (approximately 1/10 of the blood volume) was added to the preparation. The majority of normoblasts showed distinct movements typical of life.

Observation period 24 hours. Ten normoblasts were studied and depicted. Vigorous movements in the Golgi apparatus were noticed in all the normoblasts. Already at the beginning of the observation

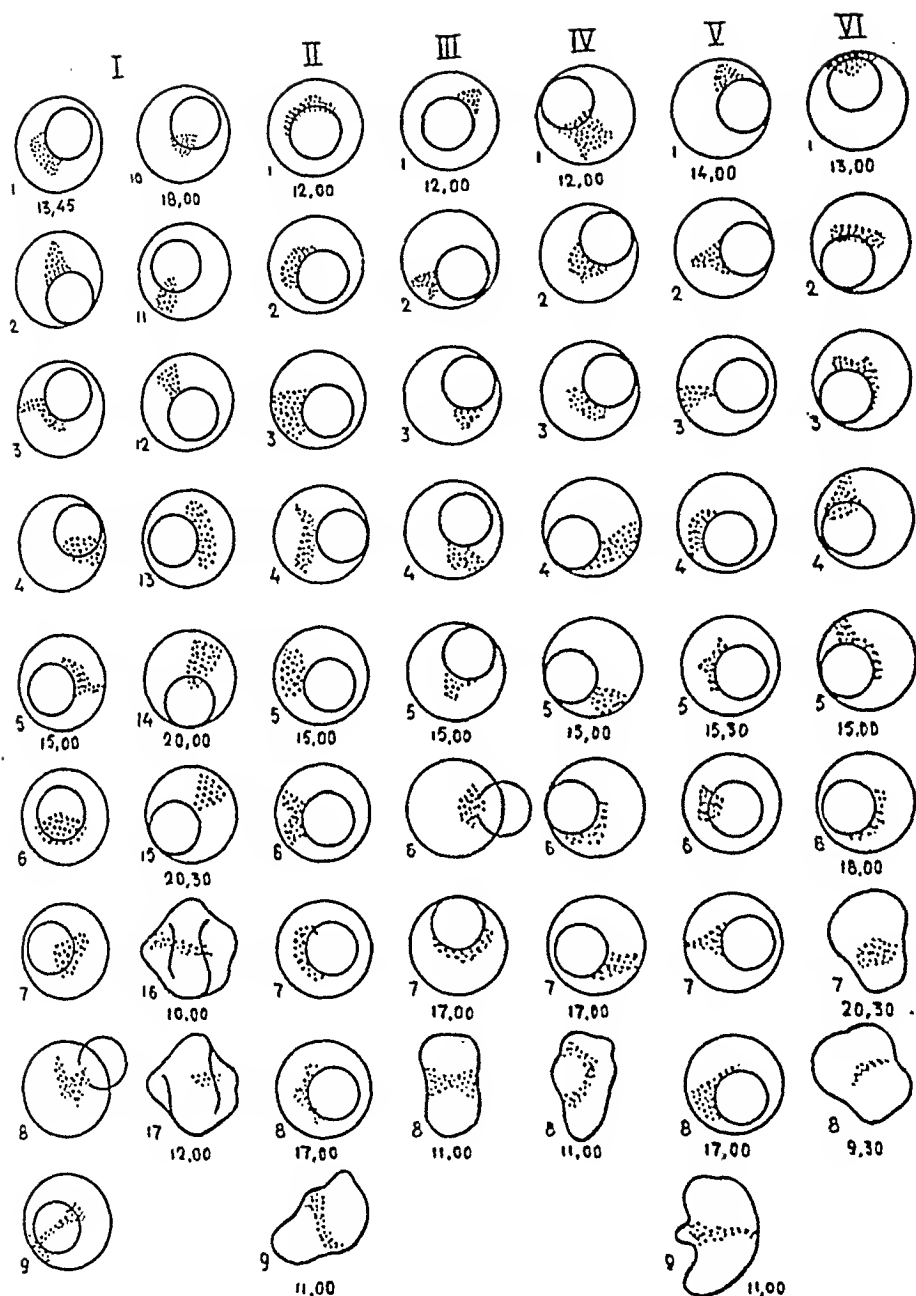


Fig. 1. Diagram showing the development of those normoblasts which in the present investigations lost their nuclei. Changes in shape and position of the Golgi apparatus are seen clearly; the dislocation of nuclei is also evident (1, 8 and III, 6). The transition from the round shape of the normoblasts to the shilus form of the reticulocytes is not so marked as indicated in the picture.

period one of the nuclei had been dislocated but was pulled back again shortly. After 2 ½ hours' observation a second nucleus was dislocated out. It remained outside for 45 minutes. ½ hour later the same phenomenon was noticed in a third normoblast. The nucleus was drawn in again after about 15 minutes.

Unfortunately, on this occasion the observations had to be discontinued after 5 hours. By that time all the nuclei were distinctly discernible but the nuclear structure was in all the cells extremely vague.

On the following morning when the observations were re-continued, 21 hours from the start, 4 cells had lost their nucleus (see fig. 1, II—V). 3 of them were then typical reticulocytes in all respects. In the fourth the granules were arranged about an optically void area the size and shape of a nucleus, but no trace of nuclear structure or remnants of membrane was seen (see fig. 1, IV). No extruded nuclei were noticed in the surroundings. After 24 hours the picture was exactly the same. No signs of life were observed anywhere. Dislocation of the nucleus had occurred in only one of the 4 cells (see fig. 1, III). Two cells from which the nuclei had been dislocated and drawn back again, retained them until the end of the observation.

Thus, out of 10 normoblasts, 4 or at least 3 had lost the nucleus and been transformed into more or less typical reticulocytes. Unfortunately, the actual process could not be observed in this case. Many facts, however, indicate that the nucleus disappeared through intracellular dissolution.

*Case IV.* Malignant lymphogranulomatosis was diagnosed. Reticulocyte count 1.2 per cent. The specimen was intended for a different purpose, but as the preparation proved to contain fairly abundant normoblasts it was examined with regard to the development of these cells. No foreign material was added to the preparation. The majority of normoblasts showed distinct signs of life. Observation period 24 hours. 10 normoblasts were studied and depicted. The Golgi apparatus in all the cells showed fairly rapid changes in shape and position. After 2 hours one of the nuclei was dislocated out for a short period. The nuclear substance in all the cells became gradually more indistinct. In one cell the nucleus disappeared after 7 hours' observation (see fig. 1, VI). It could not be detected despite repeated observation. By this time the cell had the appearance of a reticulocyte with abundant granules.

The observations were suspended for the night and recontinued 20 hours after the start. The cell that had lost its nucleus had then been stripped of most of its reticulocyte granules. Only a few granules rendered it distinguishable from a mature erythrocyte. All the

rest of the normoblasts retained their nuclei. No signs of life were noticed in the preparation. After 24 hours the picture was, on the whole, unchanged.

Thus, in this experiment 1 out of 10 normoblasts had lost its nucleus. The case is remarkable in two respects. Firstly, every possibility of extrusion of the nucleus may be ruled out, with the exception, perhaps, of a rapid, explosion-like process. Secondly, it is obvious that the addition of liver or folic acid is not an essential condition for intracellular dissolution of the nucleus in native preparations.

These observations tend, naturally, to bear out the conception of the intracellular dissolution of the normoblast nucleus. Insofar as they are characteristic of the physiological mode of disappearance of the normoblast nucleus, they rule out every possibility of platelet formation from nuclei of normoblasts and provide the counter-evidence called for by SCHILLING.

It is to be regretted that it has been impossible in the present investigation to represent photographically the series of development of the normoblasts. Unfortunately, the living cells are too sensitive to light to allow the removal of the protective filter for the purpose of photography. If this were done, the cells would die or at least be injured severely. In fig. 1 I have therefore tried to sketch the development of the normoblasts which in the above-described observations lost their nuclei.

Several questions may naturally arise as to the influence of the experimentally produced conditions which are foreign to the cell. Does the transfer from the blood-stream to the native preparation tend to cause a possibly non-physiologic intracellular dissolution of the nucleus? Is this possibly a result of the patient's illness? Would the dissolution of the nucleus have any connection with the addition of liver and folic acid?

More detailed future investigations will give the definite answers to these questions. Some facts, however, may be cited here, which indicate that this is the normal process. Only a fractional number of the normoblasts lost their nucleus. Those that did, assumed an appearance which agreed completely with that of the reticulocytes, that is, those elements of the blood which we have learnt normally to regard as transitional forms between normoblasts and mature red blood corpuscles. The

dislocation of the nucleus, typical of the development and seen only in more advanced normoblasts, is in many cases observed in immediately fixed normal blood. The lively motion of the granules, typical of the cytoplasmatic drop substance of the living cell, seems to indicate that the studied normoblasts were intact cells.

Although I am fully aware of the fact that this argument is of an indirect nature, I believe, nevertheless, that future research will succeed in demonstrating that the above-described development is typical of the normal red blood corpuscle. It is obviously assumed that only the most advanced normoblasts would reach further development under the conditions produced in the present experiments. It must be remembered that the native preparations are completely cut off from their natural supply of nutrition. Under these conditions it is natural that only a few normoblasts are able to grow into reticulocytes. The addition of liver extract and folic acid, respectively, to the preparation in cases II and III may perhaps have contributed to the success of the experiments in that it supplied some material essential to the development of the normoblast. This statement does not imply that this important material need necessarily be the specific antianemic component of these fluids. Two factors render it difficult to apply these methods of examination to native preparations from normal cases, namely, the difficulty in finding a sufficient number of more advanced normoblasts within a reasonable area to be examined, and the fact that the development is noticeably slower as compared to the above-described cases.

As pointed out by I. WALLGREN, a larger or smaller number of free normoblast nuclei occur in most native preparations. They remain unchanged during the whole observation period and do not increase in number. They show no properties characteristic of platelets. SIMMEL, on examining normoblasts in dark-ground illumination, tried without success to separate the nucleus from the rest of the cell by means of a »micromanipulator». As already mentioned, SCHILLING (371) in a small number of cases, believed that he observed formation of platelets from the interior of red blood corpuscles. The observations were



made with native preparations and in dark-ground illumination. Despite the fact that my attention was fixed on the possibility of this mode of formation I was never able to make a similar observation.

The material is not large. Nevertheless, it indicates a clear and interesting course for future investigation. To study this problem on a large scale, however, considerable technical resources and a large staff of collaborators would be required.

### c. *Cabot's ring bodies.*

When CABOT, in 1903, discovered the ring-shaped bodies in red blood corpuscles which have been named after him, he regarded them as remnants of nuclei. This conception of the nature of the rings has since been accepted generally. A few investigators, however, especially of late years, have held different views. RUSROW considered that the rings were artefacts. SCHLEICHER, recently, advanced the opinion that the rings were laboratory products consisting of denaturated proteins. SCHILLING (381), ISAACS and TSAMBOULAS & MALIKIOSIS, in a few cases, observed rings in nucleated red blood corpuscles. In the literature of textbooks, however, most authors still regard Cabot's rings as remnants of the nucleus or the nuclear membrane.

In an attempt to find further evidence for the intracellular dissolution of the normoblast nucleus, the hypothesis was brought forth that nuclear structure may be observed in Cabot's ring bodies on examination in dark-ground illumination. It may be possible that in the process of dissolution, the normoblast nucleus would pass a stage where the nuclear substance did no longer stain, although it would still be observable in dark-ground illumination.

No definite proofs were obtained to bear out this hypothesis. Numerous red blood corpuscles with Cabot's ring bodies were examined. A few cells seemed to show a distinct nuclear structure within the ring. In one case the picture resembled exactly that of a more advanced normoblast. The nucleus was indeed framed in by a structure altogether reminiscent of a Golgi

apparatus. In many cases there was a suggestion of an indistinct nucleoid granular substance within the ring, but in numerous other cells the area surrounded by the ring was optically void.

These observations do not justify any definite conclusions. It has been impossible to judge the nature of the rings or the manner in which these red blood corpuscles lose their nucleus. Cabot's ring bodies may be of different appearance both in transmitted light and in dark-ground illumination. The possibility that such rings may arise in different ways should not be ruled out entirely.

This chapter would not be complete without a review of some other studies on the development of the mature red blood corpuscles. BOSTRÖM and JACOBSEN & PLUM propose that all erythrocytes arise from the cytoplasm of the normoblasts through constriction. SCHULTZ & BUDING, HABELMANN, I. WALLGREN (472) and RALPH have observed the formation of erythrocytes from constricted fragments of megaloblasts and normoblasts in cases of pernicious anemia. WALLGREN was never able to observe a similar formation of erythrocytes from normoblasts in normal cases and regards, therefore, this mode of formation as pathologic.

I have not had the opportunity of studying the formation of red blood corpuscles in advanced and non-treated cases of pernicious anemia. On the other hand, in the experiments on which the present work is based, I have never seen the formation of red blood corpuscles through constriction.

## 8. Recapitulation of Schilling's experiments with rapid fixation.

Already in 1911 V. SCHILLING was found among the followers of the theory according to which platelets are derived from the normoblast nuclei. He was obviously aware of the flaws in his argumentation and continued to work on this problem. In 1918 he published a communication (373) by the title of

*Die Lösung der Blutplättchenfrage . . .* By means of the method of rapid fixation described below, he was able to obtain pictures which he believed represented all transitional forms from normoblast nuclei to platelets. Later he has been more cautious in his interpretations of these observations. BRIEGER made an investigation according to the same methods and expressed his doubts as to SCHILLING's interpretation. »In der jetzigen Form kann nie von einer Lösung der Plättchenfrage — eher von einer Verwicklung — die Rede sein«. SCHILLING's opinion was criticized sharply also by DEGWITZ.

Many other investigators have made observations which disprove the theory that platelets are derived from red blood corpuscles. Most of these observations have been reviewed in the historical survey. In addition may be mentioned here the interesting observations made by TÖTTERMAN, recently, with regard to the correlation between the uric acid values, the number of reticulocytes and the number of platelets in the blood in pernicious anemia in remission. He found that there was no definite correlation between the increase in the number of platelets and that of reticulocytes. The time for the increase in the number of platelets varied considerably in many cases. TÖTTERMAN considered that his observations were a strong argument against platelet formation from normoblast nuclei.

In order to make a thorough study of the problem of the origin of platelets, SCHILLING's illusive pictures have been re-examined in dark-ground illumination. This author's detailed instructions for the production of the preparations have been followed closely.

In his communication SCHILLING (373) describes clearly his method of rapid fixation. A brief account of it will be given here. Blood from a cubital vein is allowed to flow through a cannula carefully coated with paraffin, directly into a Petri dish filled with »Dominici fixative«. By shaking, the drops of blood are distributed evenly in the dish. After the blood has been allowed to stand for several hours in the fixative, the content of the dish is centrifuged, the solution of mercuric chloride is poured off and replaced by distilled water. The centrifugate is washed thoroughly a couple of times and the water is poured off, where-

upon some of the brownish-red suspension is spread with a platinum loop over an object glass which has been coated with a thin layer of albumen-glycerin. When the preparation has dried, it is re-fixed with methyl alcohol. The remains of mercuric chloride are removed in tincture of iodine and 0.5 % solution of sodium thiosulfate. After repeated washings with water staining according to Giemsa is done. When completely dried the preparation is embedded in Canada balsam and the examination is done under cover glass with oil immersion.

With the use of this method it was found easy to obtain pictures which harmonized completely with those published by SCHILLING (see fig. 11). In transmitted light platelet-like elements were seen everywhere in the preparations. They lay close to erythrocytes and a small number of them were projected partly or completely towards the erythrocyte. A few of them occurred quite freely.

Already with this method of examination, thus, with largely the same optics as those probably used by SCHILLING, the suspicion arose that the elements involved may be platelets which adhered to the erythrocytes. In many cases the hyalomere of the platelet was protracted between the granulomere of the platelet and the surface of the erythrocyte (see fig. 11). In some cases the hyalomere was distinctly projected towards and seemingly attached to 2—3 erythrocytes simultaneously: In those cases where the platelets were projected towards the erythrocyte the two elements gave the distinct impression of lying on different levels.

In order to ascertain the nature of these pictures the preparations were examined in dark-ground illumination.

The platelet-like bodies were detected without difficulty (see fig. 12). They were found to have exactly the same structure as platelets, that is, various-sized granules of the same size as in platelets appeared beside dark granules. Thus, the structure was altogether different from that of the normoblast nucleus in general and the more advanced normoblast nucleus in particular (see page 52).

If SCHILLING's interpretation were accurate, one would expect to see approximately as many Golgi apparatus as plat-

lets. The Golgi apparatus would either remain in the red blood corpuscle which would thus be a reticulocyte, or, as a result of the method used, accompany the nucleus or escape together with it. I have never been able to find any remnants whatsoever of the Golgi apparatus in the cells which according to SCHILLING would give rise to platelets. Nor have I observed any such remnants accompanying platelets. There is no evidence indicating that the Golgi apparatus would escape separately, as no remnants of them were found in the blood-plasma despite the rapid fixation. The final possibility is that the Golgi apparatus may escape before the nucleus. This, however, has never been the case in I. WALLGREN's and my examinations of bone-marrow.

Thus, through SCHILLING's method of rapid fixation the platelets appear for some reason or other to become attached to red corpuscles. The method, however, does not seem to furnish any support whatsoever of the theory that platelets are derived from the nuclei of normoblasts. Thus, one of SCHILLING's most important arguments has lost its weight.

## 9. Recapitulation of Ørskov's experiments with poisoning.

During the last decade the Danish scientist ØRSKOV has advocated energetically the theory that platelets originate from the nuclei of normoblasts. As pointed out in the general history, his opinion differs from SCHILLING's with regard to certain details.

ØRSKOV's views were borne out principally by studies on formalin-fixed blood from rabbits which had been given intravenous injections of large doses of phenylhydrazine as well as lead chloride. In these experiments ØRSKOV claims to have observed all transitional forms from nuclei of normoblasts to platelets.

ØRSKOV considers that his methods may be applied without difficulty in subsequent investigations. I have tried to recapitulate his experiments, following closely his instructions.

The result was unsatisfactory despite the fact that approximately 20 preparations were produced in connection with 4

different poisoning experiments on rabbits. Only one preparation showed an unusually large number of platelets situated close to or on (underneath) red blood corpuscles (see figs. 13 and 14). Thus, this preparation showed a picture which on the whole agreed with those obtained in my experiments with SCHILLING's rapid fixation. This preparation was sent to ØRSKOV who considered, however, that it did not agree with the preparations produced by him previously. The interpretation of ØRSKOV's pictures must therefore be left open. I should not be surprised, however, if these pictures as well as SCHILLING's, were conditioned by an increased tendency of the platelets to adhere to the red blood corpuscles. Against the background of all the observations described in the present work I cannot believe in ØRSKOV's interpretations of his findings.

#### IV. Discussion and conclusions.

The comprehensive literature on the question of the origin of platelets has been discussed elsewhere (see page 28). The historical survey shows that WRIGHT's theory, according to which platelets are derived from the megakaryocytes, has attracted most followers. It was pointed out, however, that the evidence is indirect. SCHILLING's and ØRSKOV's opinion that extruded nuclei of normoblasts give rise to platelets, has also gained some support and conclusive counterevidence has been lacking. Thus, the origin of platelets has not been considered a settled question. Other theories seem to be more or less forgotten.

The structure of stained platelets in smears has previously been studied closely in transmitted light and compared with the structure of other blood and bone-marrow cells. Comparisons have been made between the stainable granules of platelets and megakaryocytes. Opinions differ. It appears, however, that all transitional forms from megakaryocyte structure to platelet structure have been observed.

The similarities in the structure of platelets and megakaryocytes are much more evident in dark-ground illumination, in which, according to I. WALLGREN, a different kind of granules, the pale drops, are observed more easily. Both platelets and megakaryocytes have pale granules or drops of different size which varies within the same limits in the two elements. They share this common feature with the monocytes, whereas all the other white blood corpuscles have a different structure which may be distinguished readily from that of platelets. These similarities are observed without difficulty in fixed and stained preparations as well as in native preparations. They seem to

give further support to WRIGHT's theory, although of an indirect nature. At the same time these comparisons are arguments against the formation of platelets from white blood corpuscles, with the exception, however, of the monocytes.

With regard to the changes shown by platelets and megakaryocytes in native preparations, the similarities observed in the present investigation are striking. The typical rims and blisters common to platelets and megakaryocytes, have not been demonstrated in any other blood and bone-marrow cell. Although not quite constant, these changes seem to have been observed sufficiently frequently to be used as a strong argument in favour of WRIGHT's theory.

The same may be said about the accumulation of fibrin which has been observed about some megakaryocytes and which is typical of platelets. The finding has been recorded only for 20 per cent of the megakaryocytes, but the number appears to be sufficiently large to justify the ruling out of the possibility of chance, particularly as other blood and bone-marrow elements practically never show accumulation of fibrin. The question arises whether the accumulation of fibrin may be due to platelets which frequently surround the megakaryocytes. This does not seem to be the case, however, as the accumulation of fibrin about the giant cells of the bone-marrow has occurred quite independently of platelets and platelet-like elements.

It should be mentioned particularly that the monocytes have never shown rims or blisters of a kind typical of platelets. Nor has any accumulation of fibrin been observed about monocytes. Thus, the monocytes appear to have much less in common with platelets than the megakaryocytes.

Studies in dark-ground illumination of the development of the normoblasts in native preparations reveal scarcely any fact indicating a genetic relationship between normoblasts and platelets.

The difference in the structure of normoblasts and platelets is considerable. In the more advanced normoblast nuclei the



structure becomes more indistinct and the difference as compared to the structure of platelets is thus increased. Just these more advanced normoblast nuclei, however, would naturally be expected to give rise to platelets.

The free normoblast nuclei which are not seldom seen in native preparations, have a similar structure to the intracellular nuclei. They remain unaffected during long periods of observation and show no changes typical of platelets.

The nuclei of normoblasts would not be capable of producing platelets unless nuclei or fragments of them were extruded from the cells. No extrusion of the nucleus has ever been observed in studies of the development of normoblasts in native preparations. In these examinations the nucleus has in every case disappeared through intracellular dissolution. A series of circumstances have been described elsewhere (see page 59) which in all probability indicate that intracellular dissolution is the normal mode of disappearance of the nucleus. It seems, therefore, obvious that the nucleus itself is not capable of producing platelets.

The nucleus of the normoblast is framed in by a structure which is built up in a manner characteristic of different cell structures. According to I. WALLGREN it is composed of dark and pale drops intimately mixed. WALLGREN considers that this structure should be designated as a Golgi apparatus and that it coincides with the substantia granulo-filamentosa of the reticulocytes. With regard to size, the granules of this Golgi apparatus resemble markedly the platelet granules, although the experienced observer should always be able to distinguish them. Irrespective of structural dissimilarities, it seems, however, impossible that this structure would be capable of producing platelets. During the observations of the subsequent development of this structure single granules were seen, in every case, to be extruded, until a mature erythrocyte had been formed. These observations agree well with the previously known series of development of reticulocytes. All transitional forms have been observed, from young cells with abundant vital-stainable substance to old cells with only sparse remnants of substantia granulo-filamentosa.

Thus, as platelet formation from the nucleus or the Golgi apparatus of the normoblasts seems to be ruled out, a new interpretation must be given to SCHILLING's and ØRSKOV's observations. It has been established without difficulty that through SCHILLING's method of rapid fixation the platelets tend to adhere to the surface of the erythrocytes, which has given the impression of different stages of platelet formation from red blood corpuscles. It has been very difficult to recapitulate ØRSKOV's experiments and I have not felt justified in drawing any definite conclusions. I believe, however, that also with regard to ØRSKOV's pictures the explanation is that platelets adhere to red corpuscles.

Thus, the long-lived question of the origin of platelets appears to approach a final solution. The numerous arguments in favour of WRIGHT's theory are, certainly, still of an indirect nature. All the previous studies, however, augmented by the results of the observations reported in the present communication, represent an accumulation of evidence which seems to have only one explanation, namely, that the megakaryocytes are the mother cells of platelets. The actual formation of platelets in living bone-marrow cultures remains to be observed. This final piece of evidence will probably be presented as soon as the satisfactory physiologic conditions for a normal function of megakaryocytes in bone-marrow cultures have been produced.

Platelets are derived, principally, from the cytoplasm of the megakaryocytes. I am not able to judge whether the nucleus also takes part in the formation. The observations on which the present work is based have not been suited to throw any light on this detail.

The present studies of megakaryocytes are based on examinations in normal bone-marrow. Thus, they give no proofs as to the origin of platelets in pathologic cases. In this connection cases of excessive demand for platelets are brought to mind. The possibility should not be ruled out that other cells than the megakaryocytes are capable of producing platelets, vicariously, to supply the need. Monocytes (or other monocytoid cells)

## V. Summary.

After a brief introductory survey of the earliest results of the investigations on platelets, the author gives a review of all the essential contributions to the question of the origin of platelets, within the frame-work of the available literature. It is established that the majority of present-day investigators favour WRIGHT's theory, according to which platelets originate from the megakaryocytes. Only the conception that platelets are derived from the nuclei of normoblasts is nowadays mentioned as a possible alternative.

The author's investigation is based on studies of platelets and megakaryocytes and of the development of red blood corpuscles in dark-ground illumination. Microscopic studies of native preparations on the heating table play an important part.

A detailed account is given of I. WALLGREN's new opinion, according to which the living cell substance is built up of intimately mixed dark and pale granules or drops in a structureless ground-substance. This conception seems to open up new prospects in the matter of judging numerous cytoplasmatic and nuclear structures. Most of the observations on which WALLGREN bases his views have been confirmed in the present investigation.

Platelets appear to be built up according to the principles of WALLGREN's double drop system. It is emphasized particularly that the pale drops are of different size, by which platelets are distinguished from most blood and bone-marrow cells.

No signs of life have been recognized in platelets. It has long been known that pseudopod-like rims and protrusions and

blisters with granules in Brownian motion are formed on the platelets. It has been demonstrated that these changes are not to be regarded as manifestations of life. In experiments with magnesium sulfate, staining with brilliant-cresyl-blue in high concentrations and the addition of potassium cyanide to the preparations the changes in the platelets have occurred in the usual manner. In all these cases possibilities for true manifestations of life have been absent.

It has been found that platelets in native preparations extrude single granules. As it is impossible to distinguish these granules in the blood-plasma from hemokonia, the question of the origin of hemokonia is discussed briefly.

The term pseudoplatelets is defined and the origin of these elements is discussed. The importance of not confusing true platelets with pseudoplatelets is emphasized in different contexts in the present communication.

The megakaryocytes also appear to be built up of dark and pale granules or drops. Similarly to platelets, the megakaryocytes have pale drops of different size. The size of the pale drops varies within the same limits as in platelets.

In stained preparations examined in transmitted light the granules of the megakaryocytes are in many cases seen to accumulate in platelet-sized areas, whereas the examination of the same cell in dark-ground illumination reveals no such phenomenon. This seems to agree well with I. WALLGREN's observation that the granulomere of platelets appears noticeably larger in dark-ground illumination than in transmitted light, in the examination of stained preparations. This seems to indicate that there would be present at the periphery of platelets and of the above-mentioned megakaryocyte areas dark granules which, as distinct from most dark granules, do not stain.

With the exception of a few uncertain cases, no manifestation of life in the form of movements of granules or changes in shape typical of life, has been observed in the megakaryocytes. Similarly to platelets, most megakaryocytes (67.5 per cent) develop blisters of exactly the same nature as the platelet blisters. A small number of megakaryocytes show blisters up to the size of leukocytes and filled with Brownian-moving granules.

Distinct accumulation of fibrin has been observed about 20 per cent of the megakaryocytes.

No constriction of fragments of the cytoplasm of the megakaryocytes has been observed directly. In many cases, however, the megakaryocytes are found to be surrounded by cytoplasm-like elements. It has not been possible to decide whether these elements were pinched off fragments of cytoplasm or agglutinated platelets. In one case a seemingly badly injured megakaryocyte was by several neutrophile granulocytes stripped of all its cytoplasm, which was split off and in parts carried off in platelet-like fragments.

In a comparative study the author points out the remarkably great similarities between platelets and megakaryocytes, which are noticed on examinations in dark-ground illumination. The considerable dissimilarities between platelets and all other blood and bone-marrow cells, on the other hand, are also stressed. Structurally, the monocytes are an exception in this respect.

Also the nucleus of the normoblast, according to I. WALLGREN, has a structure which is built up of the said double drop system. Already the pale drops in the nucleus of the less advanced normoblast are smaller than the pale drops of platelets. As the normoblast develops, the structure of the nucleus becomes more indistinct. Finally, the nucleus appears almost optically void in dark-ground illumination. Close to the nucleus is seen a structure which is built up according to the principles of the double drop system and which apparently should be designated as a Golgi apparatus. It coincides probably with the substantia granulo-filamentosa of the reticulocytes.

In his studies on the cytology of the red blood corpuscle I. WALLGREN, on one occasion, was able to witness the intracellular dissolution of the nucleus in some normoblasts in native preparations. In my investigations intracellular dissolution was observed in 7 out of a total of 23 normoblasts kept under observation, from 3 patients. Many facts indicate that this is the natural mode of disappearance of the nucleus. No extrusion of normoblast nuclei has ever been observed, nor the formation of erythrocytes in normal cases through constriction of the cytoplasm of the normoblasts.

Cells with Cabot's ring bodies have been studied, but in these observations no definite evidence for intracellular dissolution has been obtained.

SCHILLING's experiments with rapid fixation have been recapitulated. The author is not able to agree with SCHILLING's interpretation of the pictures obtained. This method appears to cause adherence of platelets to the red corpuscles. Platelet formation from normoblast nuclei must be ruled out, partly as no equivalence to the Golgi apparatus can be detected anywhere. Attempts have also been made to recapitulate ØRSKOV's experiments with phenylhydrazine-lead-poisoning, but difficulties in producing satisfactory preparations have rendered it difficult to draw any definite conclusions. The interpretation given to SCHILLING's pictures, however, may probably be applied also to ØRSKOV's preparations.

A discussion on the observations made concludes the work. The arguments presented in support of WRIGHT's theory are new and noteworthy although of an indirect nature. Platelet formation from megakaryocytes remains, finally, to be observed directly in bone-marrow cultures. All other theories are discarded as unacceptable. In this respect special consideration has been paid to the fact that the normal intracellular dissolution of the normoblast nucleus seems to render it incapable of producing platelets. Thus, the megakaryocytes appear to be the natural mother cells of platelets. Although the possibility should not be ruled out entirely that other cells may, vicariously, give rise to platelets in pathologic cases, this mode of formation has not been borne out by any conclusive evidence.

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(The abbreviations follow, on the whole, the system applied in Index Medicus.)

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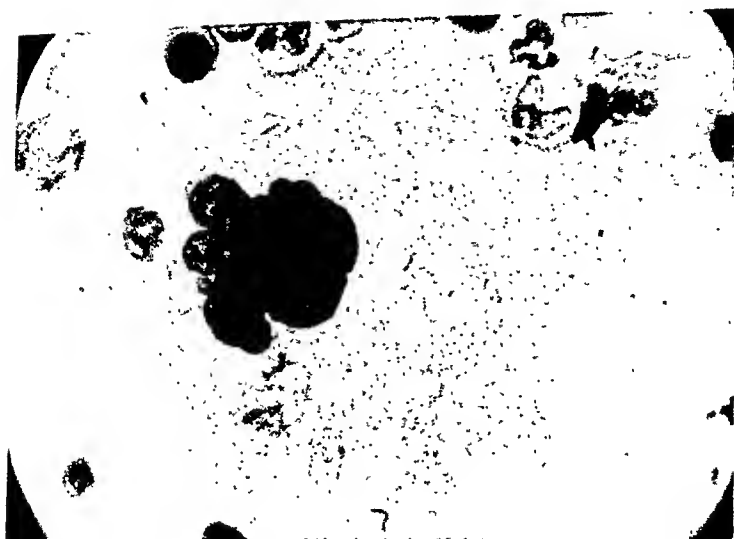


Fig. 2. Megakaryocyte in transmitted light. May-Grünwald-Giemsa.  
Approximately 1250  $\times$ .

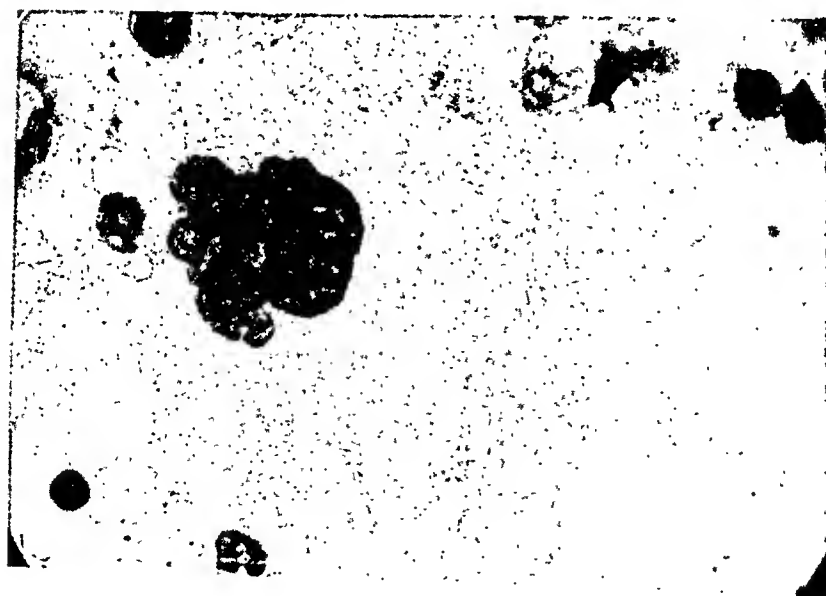


Fig. 2 a.

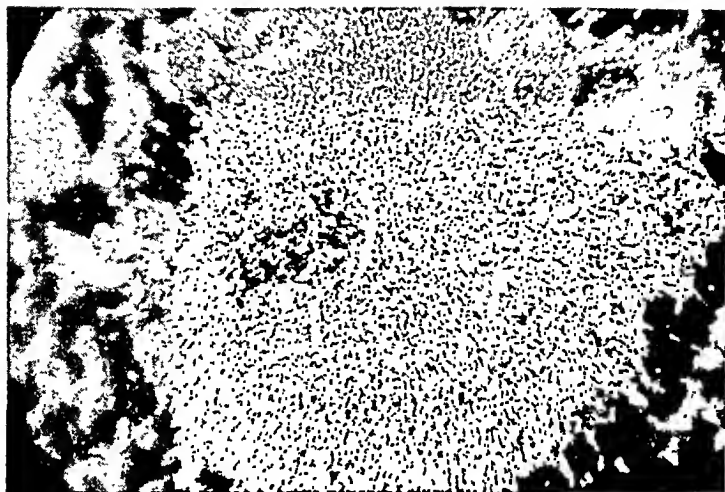


Fig. 3. The same stained megakaryocyte with the use of dark-ground illumination. The pale granules are seen clearly everywhere and between them the dark stained drops. Approximately 1250  $\times$ .

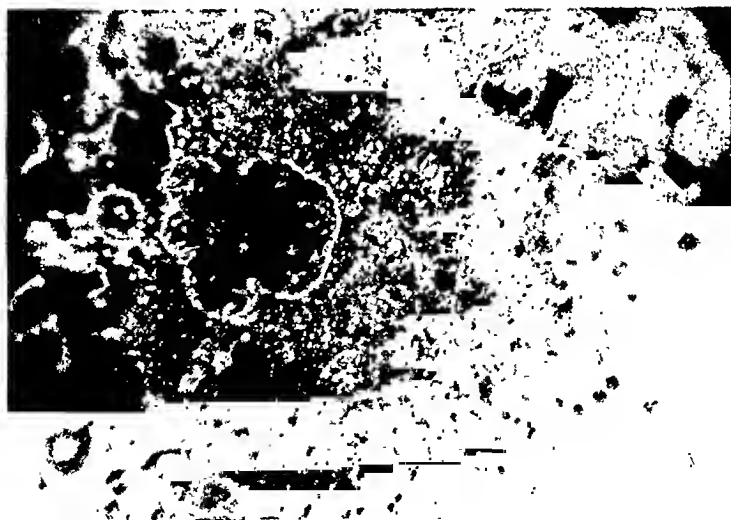


Fig. 3 a.

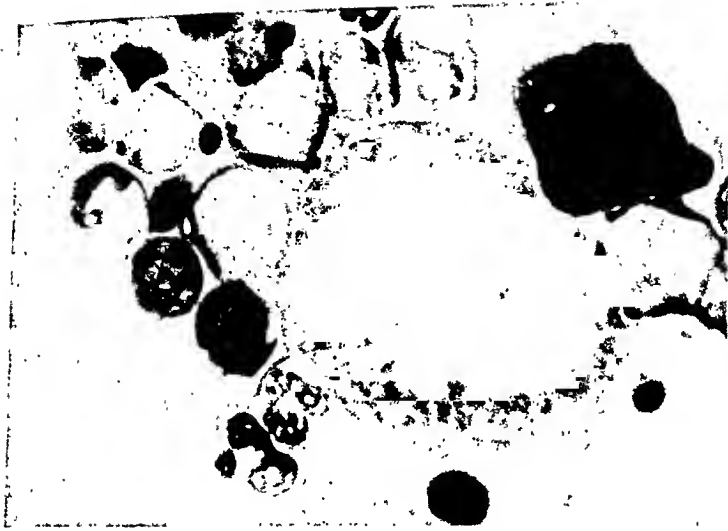


Fig. 4. Megakaryocyte with the cytoplasm largely divided into platelet-sized areas. May-Grünwald-Giemsa. Approximately 1250  $\times$ .

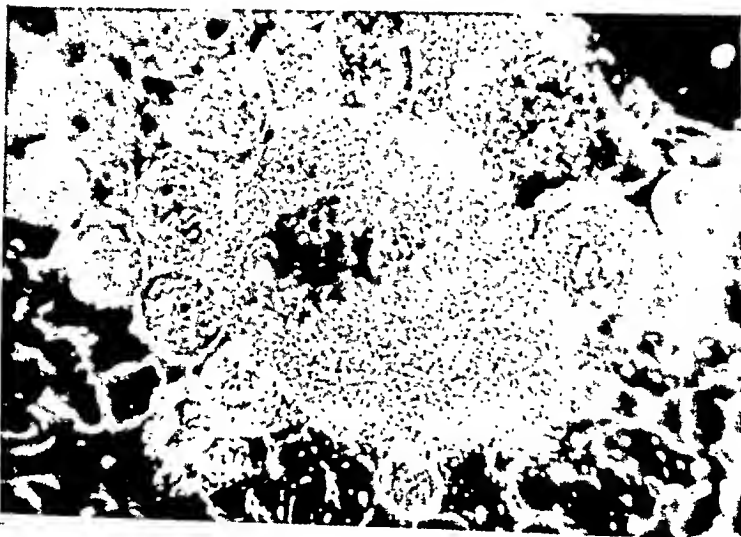


Fig. 5. The same cell in dark-ground illumination. The areas are not outlined with this illumination. Approximately 1250  $\times$ .



Fig. 6. Megakaryocyte with the cytoplasm divided into areas. Probable beginning of platelet formation seen at the top of the picture. May-Grünwald-Giemsa. Approximately 1250  $\times$ .

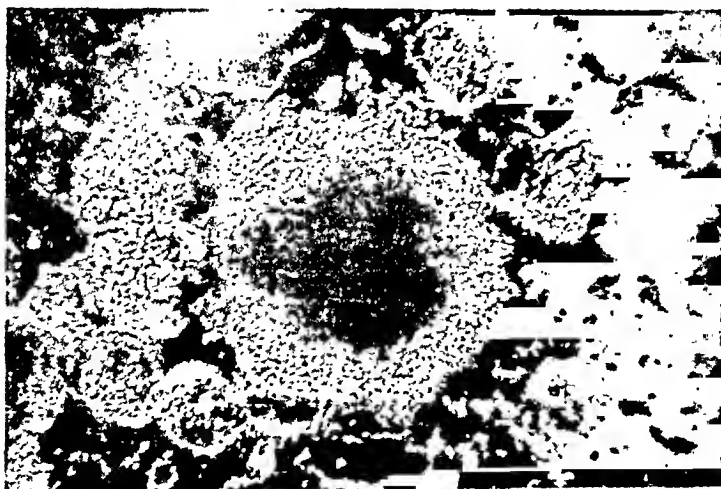


Fig. 7. The same cell in dark-ground illumination. The areas are not outlined. The new bodies are only partly distinguishable. Approximately 1250  $\times$ .



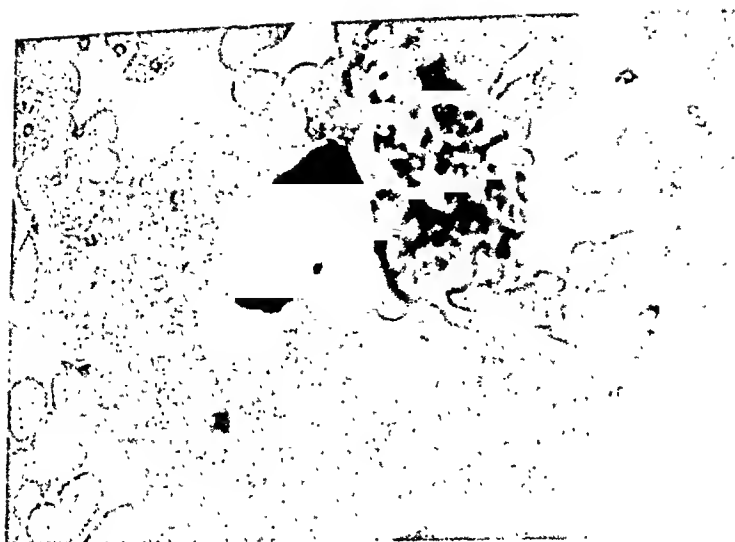


Fig. 8. Megakaryocyte and agglutinated platelets. Incidental contact, perhaps conditioned by common agglutinability. May-Grünwald-Giemsa. Approximately 1250  $\times$ .

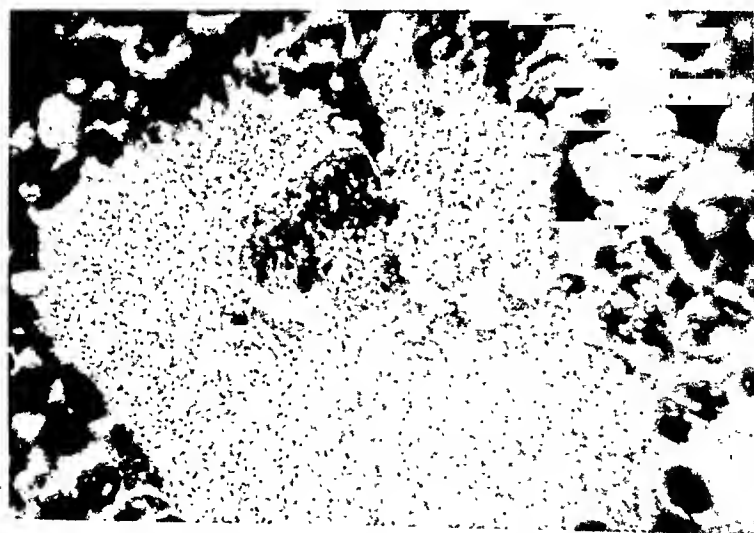


Fig. 9. The same cell and clump of platelets in dark-ground illumination. The similarity in the structure of the megakaryocyte and the platelets is evident. May-Grünwald-Giemsa. Approximately 1250  $\times$ .

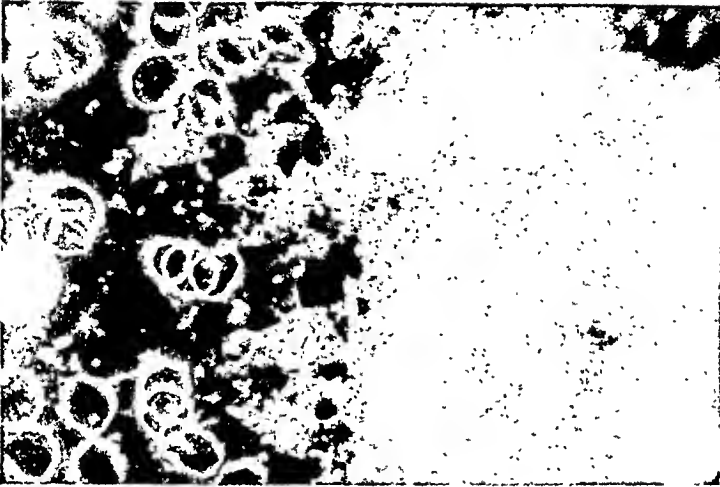


Fig. 10. Native preparation in dark-ground illumination. The entire right side of the picture is taken up by a portion of a megakaryocyte. The white rings to the left are erythrocytes. Between the megakaryocyte and the erythrocytes are seen a number of dark spaces. These are blisters formed from the cytoplasm of the megakaryocyte. The granules in the blisters are not delineated on account of the long exposure. Approximately 1250  $\times$ .



Fig. 11. Part of a Schilling preparation. The platelet indicated by the arrow adheres to three red corpuscles. Approximately 1900  $\times$ .



Fig. 12. The same part in dark-ground illumination. Approximately 2500  $\times$ .

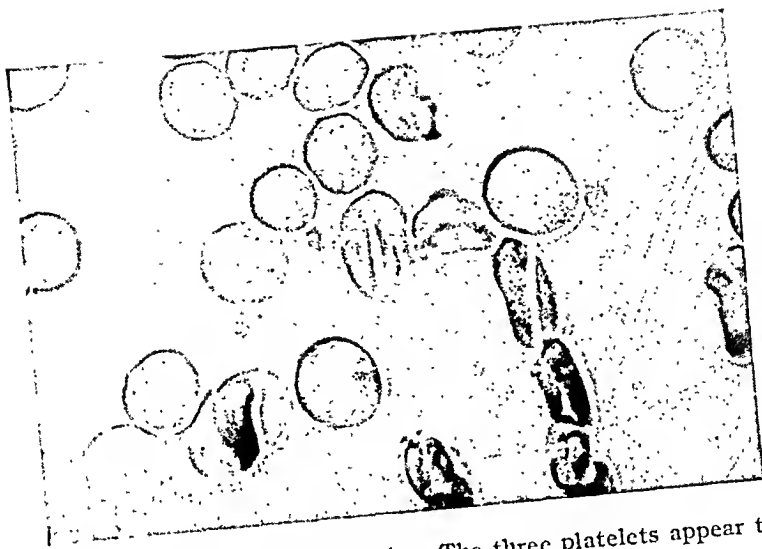


Fig. 13. Part of an Ørskov preparation. The three platelets appear to adhere to red corpuscles: Approximately 1900  $\times$ .

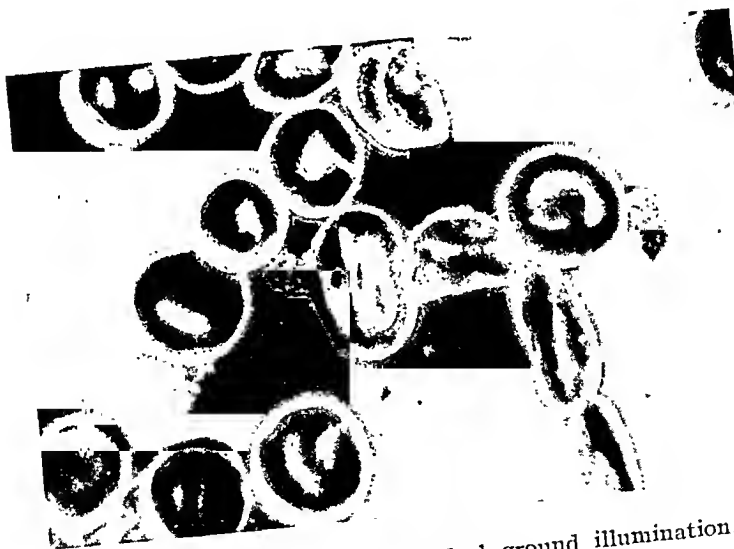
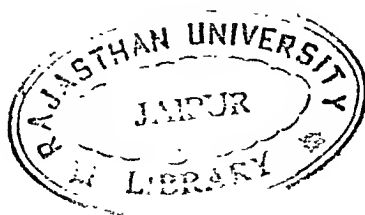


Fig. 14. The same part in dark-ground illumination. Approximately 2500  $\times$ .









- D. 1. Cases with non protein nitrogen  $> 40$  mg per cent or  
urea nitrogen  $> 20$  mg per cent  
2. Cases with non protein nitrogen  $\leq 40$  mg per cent or  
urea nitrogen  $\leq 20$  mg per cent
- E. 1. Cases with a duration  $> 3$  years  
2. Cases with a duration  $< 3$  years

### Retinal Changes

The classification of the retinal changes into groups 0-IV according to Keith (1933) was made on the same lines as for chronic nephritis (v. the foregoing). Groups 0-II, which represent the milder changes, were assembled into one group and compared with groups III-IV, which represented the other group with severe changes in the fundi. It should perhaps be mentioned that this classification into groups 0-IV was made on the basis of the retinal changes only and thus had no connexion with the classification of hypertension into four groups suggested by Keith, Wagener and Barker (1939) and Wagener and Keith (1939). The latter classification was based partly on the retinal changes mentioned in the foregoing, but regard was also paid to the degree of hypertension and the clinical symptoms. This classification is evidently more laborious than the simple one used by the present writer. The real reason why it could not be used is obviously that the material was too small, or rather that the cases with retinal changes were far too few for such a grouping.

In Table XXXIV all the data concerning the results of the tests in these two groups are given. The distribution of the cases in the two sexes was very unequal since very few patients showed advanced retinal changes and no calculation was therefore possible for either of the sexes separately. Even in a calculation of both sexes together the number of cases with severe retinal changes was small. It was nevertheless possible to make a calculation by using the same method as earlier (v. p. 150). It is seen that lower values



Table XXXIV. Mean (M) and standard error of the mean<sup>1</sup> (ε) of cases with essential hypertension, distributed according to retinal changes.

Determination	Retinal changes			
	0 — II		III — IV	
	Number	M ± ε(M)	Number	M ± ε(M)
Men				
Inulin clearance .....	15	100.5 ± 5.9	5	62.8
Diodrast clearance .....	15	316.9 ± 28.2	5	178.6
Filtration fraction .....	15	0.343 ± 0.025	5	0.340
Creatinine clearance .....	4	112.5	1	72.0
Urea clearance .....	8	61.1	2	31.5
Effect. renal blood flow ....	15	588.7 ± 53.9	5	327.0
Hæmatocrit .....	15	45.3 ± 1.4	5	40.8
Proteinuria .....	15	0.113 ± 0.068	4	0.125
Blood pressure { systolic ....	15	187.0 ± 7.9	5	234.0
	15	111.7 ± 5.3	5	143.0
Urea nitrogen, mg % .....	12	22.0 ± (1.7)	3	35.5
Women				
Inulin clearance .....	36	94.6 ± 3.1	6	70.5
Diodrast clearance .....	36	292.5 ± 11.6	6	199.7
Filtration fraction .....	36	0.335 ± 0.011	6	0.363
Creatinine clearance .....	10	95.1 ± (8.4)	3	114.7
Urea clearance .....	28	64.4 ± 3.8	4	42.5
Effect. renal blood flow ....	36	519.4 ± 21.4	6	349.3
Hæmatocrit .....	36	43.44 ± 0.56	6	42.50
Proteinuria .....	36	0.0222 ± 0.0070	6	0.6667
Blood pressure { systolic ....	36	215.1 ± 6.3	6	239.2
	36	120.8 ± 3.1	6	128.3
Urea nitrogen, mg % .....	31	19.88 ± 0.82	4	22.75
Both sexes				
Inulin clearance .....	51	96.3 ± 2.7	11	67.0 ± (8.4)
Diodrast clearance .....	51	299.6 ± 11.5	11	190.1 ± (25.0)
Filtration fraction .....	51	0.337 ± 0.011	11	0.353
Creatinine clearance .....	14	100.1 ± (6.6)	4	104.0
Urea clearance .....	36	63.7 ± 3.3	6	38.8
Effect. renal blood flow ....	51	539.8 ± 21.8	11	339.2 ± (46.1)
Hæmatocrit .....	51	44.00 ± 0.57	11	41.73 ± (2.54)
Proteinuria .....	51	0.049 ± 0.021	10	0.450 ± (0.244)
Blood pressure { systolic ....	51	206.9 ± 5.2	11	236.8 ± (11.3)
	51	118.1 ± 2.7	11	135.0 ± (6.5)
Urea nitrogen, mg % .....	43	20.48 ± 0.75	7	28.21

<sup>1</sup> v. foot note p. 129

The quality of the optical equipment has no influence on the determination of the mean diameter. But there is a certain difference between the mean diameters as determined in the different parts of the visual field. Even with the best optics all parts of the field of vision are not equally sharp in focus. The quality of the optics influences, on the other hand, the determination of the standard deviation which is greater with poor optical equipment.

*The importance of the time of exposure* was tested at this stage of the investigation, without one being able to find that it played any deciding role in the results. It was found, during these preliminary experiments, that it was very difficult to ensure a uniform degree of enlargement when one used an ordinary micro-camera. This difficulty was first pointed out by BOCK & JOMBRES. Besides, it soon became clear that the expenses in connection with usual microphotography would be out of all proportion. For this reason the author constructed his own camera which satisfied the demands for accuracy of enlargement, easy, cheap management, and which eliminated the possibility for errors in the exposure.

#### *The author's camera.*

The apparatus which is reproduced in figure 3 was constructed, partly based on a description by COLES. The drawing is isometric about the axes  $30^\circ$ ,  $90^\circ$ , and  $150^\circ$ .

The tube of the microscope is enclosed in a box. The inside of the box is painted black and the top is covered by a plate which can be slid back and forth without letting in the light. In the plate is sunk a focussing screen. The focussing screen, which is  $8 \times 8$  cm, is placed in the centre of the field of vision. The blood cells will therefore be projected on it. When the picture is sharply focussed the top plate is slid over to the other side. The film, which runs from the store to the receiving-film-holder, comes into the position of the focussing screen and the exposure is made. When exposure has been made, the plate is slid back to its original position and it is checked that the picture is still in focus. A small opening covered with a cardboard plate is placed over the film. Through this opening the register number of the blood film is written on the back side of the film. After the exposure the film is rolled forward until the number appears in a small window covered with red glass. The camera is now ready for a new exposure.

CHRONIC HEPATITIS							
No. 33: H. K. W. b. 6/6-65. C. no. 30/45, 30/10-11-9/1-15. 1917: Cancer ovarii operat. 1927: Cancer mammae operat. 1940: Uterus rodens, radium-treatment. At control 26/10-14 the patient was jaundiced, without knowing for how long she had been so. 29/11-16: Explorative laparotomy: Normal galiducts. Chronic hepatitis.	DATE	Hb.	R. b. c.	%	Vol.		
	1/12	9.8	3.22	28	28	28	
	7/12	10.2	3.34	29	29	29	
	12/12	11.4	3.98	34	34	34	
	21/12	11.1	3.72	31	31	31	
	29/12	-	-	-	-	-	
	4/1	10.8	3.80	32	32	32	
	15/3	13.0	4.96	40	40	40	
	12/2	13.0	5.00	37	37	37	
	19/1	12.2	4.02	31	31	31	
	11/1	11.6	4.10	31	31	31	
	2/1	12.2	4.34	34	34	34	
	28/12	12.3	4.30	35	35	35	
	21/12	10.8	4.04	34	34	34	
	14/12	12.2	4.40	34	34	34	
	5/12	11.6	4.12	34	34	34	
	30/11	12.6	4.54	37	37	37	
	23/11	12.8	4.52	38	38	38	
	15/11	10.6	3.92	33	33	33	
	10/11	11.2	4.24	35	35	35	
	3/11	11.7	4.08	36	36	36	
	1/11	12.2	4.22	37	37	37	
	15/5	12.5	4.76	36	36	36	
	11/5	12.3	4.30	31	31	31	
	18/5	12.2	4.30	32	32	32	
	24/5	10.1	3.44	30	30	30	
	31/5	11.1	4.08	34	34	34	
	6/6	10.8	4.44	34	34	34	
	13/6	11.1	4.52	35	35	35	
	21/6	11.8	4.14	36	36	36	
	5/7	12.4	4.36	37	37	37	
	9/7	11.3	3.96	33	33	33	
	18/7	10.8	3.80	32	32	32	
	25/7	11.2	3.90	34	34	34	
	1/8	11.6	4.22	35	35	35	
	9/8	11.1	3.90	35	35	35	
	16/8	10.9	4.28	33	33	33	
	25/8	12.5	4.42	34	34	34	
	28/7	12.4	4.74	34	34	34	
	4/8	12.3	4.42	34	34	34	
	11/8	11.6	4.50	35	35	35	
	17/8	11.8	4.36	35	35	35	
	25/8	12.4	4.44	35	35	35	
	31/8	12.4	4.62	35	35	35	
	7/9	10.7	3.74	29	29	29	
	13/9	11.8	4.24	31	31	31	
	20/9	12.3	4.41	35	35	35	
	27/9	10.5	3.74	33	33	33	
	4/10	11.8	4.40	32	32	32	
		1/6	12.2	4.14	29	29	29
		8/6	10.1	3.74	30	30	30
17/6		13.0	4.98	31	31	31	
29/6		12.7	4.24	34	34	34	
2/8		12.6	4.76	-	-	-	
		No. 36: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 37: K. M. W. b. 2/4-72. C. no. 830/46, 31/5-16-8/7-46. Jaundice 6 weeks before adm. 31/5: R. f.: 0.0%, 1/6: R. f.: 0.28/0.46, 3/6: A/G.: 3.4/2.8. T.K. at the time of adm. + at the time of discharge: T.: 0.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 38: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 39: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 40: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 41: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 42: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 43: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 44: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 45: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 46: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 47: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 48: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 49: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 50: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 51: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 52: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 53: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 54: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 55: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 56: J. H. W. b. 21/11-84. C. no. 1147/15, 27/7-15-18/10-15. Dyspepsia with jaundice for 4 weeks 3 month before adm. Three weeks before adm. jaundice anew. Last two weeks ascites. During the stay in hospital repeatedly performed paracentesis. 16/10: Coma hepaticum. Stors. No autopsy. 28/7: R. f.: 0.22/0.38. T.K. ++++. T.: 14/8: Liverextract 100 cm. l. m. 20/8-5/9: Aneurin, 0.025 g. l. m. daily.	1/6	12.2	4.14	29	29
		8/6	10.1	3.74	30	30	30
		17/6	13.0	4.98	31	31	31
		29/6	12.7	4.24	34	34	34
		2/8	12.6	4.76	-	-	-
		No. 57: J. H. W. b. 21/11-84. C. no. 1147/15, 27					

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So far as I can find, AFFANASIEW, 1884, was the first to advocate an erythrocytic origin. He agreed with HAYEM insofar as he regarded platelets as offsprings of the nucleus of nucleated red blood corpuscles, which, in their turn, became nucleated red blood corpuscles. This hypothesis has gained no support in the literature.

a. *Cytoplasmatic origin.*

Although the origin of platelets was already early attributed to the nucleus of the erythroblast, the opinion that platelets are detached fragments of mature erythrocytes predominated in 1890—1900. This theory was successfully upheld by ARNOLD in a series of publications. He considered that the similarity between platelets and detached portions or fragments of mature red blood corpuscles indicated strongly an erythrocytic origin. He found further support for his theory in the fact that he believed he was able to recognize hemoglobin in platelets. ARNOLD discarded, on various grounds, the theory of a leukocytic origin as well as the conception of platelets as products of precipitation. Also he criticized the theory that platelets are derived from the nuclei of normoblasts. ARNOLD's opinion was supported by BREMER, WLIASSOW, DETERMANN, FELDBAUSCH and HUBER. WLIASSOW's works were opposed by SCHERER who found no reason to believe that platelets originate from red blood corpuscles. HELM and WATSON, much later, advanced the opinion that platelets are derived from non-nucleated erythrocytes in the process of disintegration.

It should be mentioned in this connection that MOSSO, PETRONE, ALBRECHT, WERZBERG, HEDINGER and BROCKBANK believed in an erythrocytic origin, so far as I can find, without stating in which part of the erythrocyte the platelet would arise. In his investigations on the blood of the chick SUGIYAMA reached the opinion that platelets originate from megaloblasts. SPADARO (according to TOCANTINS) attributed the origin of platelets to red blood corpuscles but also to other platelets. PUCHBERGER considered that every connection between platelets and red blood corpuscles could be ruled out, on the basis of experiments with vital staining.

would then be the most obvious cells, on account of their structural similarities to platelets. These hypotheses, however, are to be regarded as pure speculations.

The conception of the normoblast nucleus as the origin of platelets, which has competed obstinately with WRIGHT's theory, may be discarded. SCHILLING's demand for an explanation of the fate of the normoblast nucleus appears to be fulfilled, as all the facts indicate that the nucleus dissolves intracellularly. All the rest of the previously popular theories on the origin of platelets have appeared extremely improbable or altogether repudiable.

At the present stage of research there remains within the bounds of possibility only one explanation of the natural origin of platelets. In all probability, if not with certainty, they originate from the megakaryocytes.

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